

nature*May 13, 1976*

What follows the squabble?

THE engineering profession in Britain has been in disarray for some time over its structure. There are fifteen separate institutions, each of which fulfils the double role of acting as a learned society and looking after the professional interests of its particular members. Ten years ago the Council of Engineering Institutions (CEI) was formed as the first move towards providing some sort of unified body that could represent engineers to government, in international circles, or just to each other; there was never any intention that the institutions should lose their separate identities as a result of these moves.

Unfortunately, the CEI has turned out to be just what its title says it is—an assemblage of institutions, each with its own pride, prejudice, president, executive secretary, council and awareness of the size of its membership, be it above or below the average. Thus the small institutions have been worried about being steam-rollered in the CEI, while the big ones have been worried that they can be outvoted. As a result, progress towards establishing a real voice for the engineering profession as a whole has been slow and marked by considerable bitterness which led late last year to the Institution of Electrical Engineers (one of the biggest institutions) giving a year's notice of intention to quit the CEI.

Maybe this threat has had a salutary effect at a time when the CEI's statutes are now being prepared for approval by the Privy Council. In the months of negotiation ahead compromises are on the cards all round, in the knowledge that the Privy Council is hardly likely to endorse a constitution to which at least one major institution objects (and the mechanical engineers seem to be equally unhappy).

Engineers can ill afford the time and emotional, even conspiratorial, energy that is being spent by their representatives in arguing over the CEI. If conciliatory gestures fail in the near future it is inevitable that demands for a public enquiry into the organisation of the engineering profession will grow, and with good cause. There are obvious attractions to handing the whole mess over to a small committee whose members have no

axe to grind, and gracefully accepting their solution. But if this is done, it is important that the terms of reference of the public enquiry should be right. What is really needed is not a convenient way of patching together a group of institutions by neatly balancing voting procedures and carefully avoiding putting a bunch of president's noses out of joint. The body that should be created is a Council of Engineers to which individuals would have a stronger sense of loyalty. Some argue that modelling such a council on the General Medical Council would be a good thing, producing a lean organisation with some lay membership, concerned with qualifications, discipline, education and so on. Certainly some bread-and-butter matters such as the nature of engineering qualifications and their interchangeability within the European Economic Community need attention, and such a body would devote much of its time to these affairs. But engineers should not pass up the opportunity to go further and use the council to provide an accessible profession-wide forum.

Engineers are often not people with the broadest vision, indeed sometimes their vision seems narrower even than that of scientists. In times of plenty this may not seem to matter too much—there will always be work available and few will question the benefits of technology. The problem surfaces only when times are hard, and it may be characterised as a loss of nerve. Technology is then not only an easy target for cutbacks but is accorded the blame for many of our woes. On the whole engineers do not seem to have come to terms very well with new moods in society, and one of the reasons for this is probably that there is not a great enough number of them prepared to read, think and talk together about the place of the engineer in society. This surely must be done on as wide a basis as possible, using the resources that all sectors of the profession can bring. While there is dissent about the CEI it cannot be done effectively. A public enquiry should at least be aware that some needs of the engineering community go beyond those of simply voting, acquiring a string of qualifications and doing a day's work. □



The difficulties facing West German science

What is the current state of science in West Germany?

Rainer Flohl reports on a situation which scientists in other developed countries may find familiar

SCIENTIFIC research in West Germany is going through a difficult period. It is suffering from financial restrictions similar to those in many other countries. More importantly, it has been affected by the structural changes in West German universities over the past few years. Indeed, after a belated advance in the mid-1960s, when it received increased government support, West German science again stands on the brink of disaster. The winner of the Nobel Prize for Chemistry, O. E. Fischer of the Munich Technical University, has asserted that West German universities are losing ground in international terms. He compares research units in West German institutions of higher learning to empty shells, and the strong teams in Anglo-Saxon countries to battleships. German scientists cannot hold their own against this kind of opposition: the once-successful *Geheimratsystem* has disappeared, and with it has gone the glamour which had attracted scientists to West Germany from all over the world.

The overthrow of that system was in fact a reaction to the growing demands of an ever more complex science. The anticipated consequences—intensified cooperation among scientists, greater “teamwork”, more criticism and self-criticism—have failed to materialise. But this failure was disguised in the early 1960s when increased financial support for science led to a certain amount of progress. Now the deterioration is apparent, though the true situation is not generally understood. There are three reasons for the deterioration: the aforesaid structural change; the overburdening of universities with teaching; and the financial crisis, which has drastically exposed the weak position of science.

The overburdening with work of teachers in higher institutions is the result of the explosion of further education, which even now continues to attract more and more students to universities. Growing numbers of university teachers were needed to instruct the students. These teachers wished not only to teach, but also—in the German tradition of “unity of research and teaching”—to carry out research for themselves. This has led to a doubling of the number of scientists in the Federal Republic over the past few

years. The means for research work have not risen proportionately, so the average research worker has less money than at any previous time. Besides this, the lecturers have had to spend more time on their teaching. Only in this way could they keep up with the rising number of students, whose education—for political reasons—appears to be more important than research work. The time available for research has been reduced because the democratisation of higher education demands too many meetings and additional administrative expenses.

The financial crisis has led to a drastic reduction in the funds available for research. Many programmes have had to be arbitrarily curtailed. In 1975 approximately 30 to 40% of allocated funds were withdrawn; there will be no change in the situation in 1976. People have accepted the change without complaining. Unrest at the universities has subsided. The only remaining tensions are in the departments responsible for teacher training; but fully qualified students have increasing difficulty in finding work, which makes them insecure. Many of them blame the so-called *Radikalerlass* rather than their own poor marks for their failure. The *Radikalerlass* was an administrative regulation designed to prevent professed enemies of the constitution—in particular members of the Communist Party—from getting jobs in the public service. Many rumours arose because of the generally clumsy and bureaucratic methods used to check public service applicants. It was said, among other things, that a signature on a pamphlet was sufficient to get an

applicant rejected. The regulation has recently been abolished, or rather administered in a different manner.

The Federal Republic has encountered these problems at a time when there were justifiable hopes of improving the effectiveness of scientific research. The two most important organisations conducting pure research in the Federal Republic—the German Research Organisation (DFG) and the Max-Planck Institutes (MPG)—had elected new Presidents who were well-qualified and open to new ideas for reform. Professor H. Maier-Leibnitz, President of the DFG, which receives about DM600 million a year from the Federal and State governments for the furthering of basic research, has a wealth of experience and success as a practical scientist: he built the Federal Republic's first experimental reactor in Garching near Munich, and it was due to his initiative that the Franco-German high flux neutron reactor was later erected in Grenoble. Not least because of suggestions from Professor Maier-Leibnitz, both instruments became focal points of research, and he became an international figure through his contribution to neutron optics. The President of the Max-Planck Institute, Professor Reimar Lüst, is also a physicist. Formerly President of the European Space Research Organisation (ESRO) and Chairman of the German Science Council, Lüst was the first man to use barium clouds for the discovery of astrophysical phenomena. The Max-Planck Institute also receives DM600 million from the Federal and State governments from funds set up to finance independent institutions.

Only time will tell whether Professor Maier-Leibnitz and the German Research Council will manage to restore science to its former position and thus unlock the creativity which is essential for successful research. The Max-Planck Institutes' first priority is to protect scientists from the dead hand of the bureaucracy. But both institutions face exceptional problems,

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Photos: PIB, Bonn

The West German Minister for Research and Technology, Hans Matthöfer (left), and the Minister for Education and Science, Heinrich Rohde

especially as the "reform" of the universities has blocked posts for the new generation of scientists, however well-qualified, since large numbers of mediocre young scientists secured life-long tenure in earlier years.

At the beginning of April the German Research Council published its *Grauen Plan* ("Gray Paper") analysing the present state of research in the Federal Republic. From its assessment of the present situation, and the many obvious deficiencies thus revealed, it derives suggestions for improving research, though it accepts that not all the ideas it puts forward can be carried out. The Paper covers the years 1976 to 1978, and is the most detailed and honest document yet compiled about German research. The main section deals with the structure of the universities and the financing of research. For the first time, moreover, scientists from universities were consulted in the drawing up of the document: out of 1,300 scientists approached for their views on the state of research in universities and the condition of their own field of work, 1,000 responded. The results of this inquiry are also included in the Paper, which in parts is more a catalogue of inadequacies than the report of an enquiry, though it should not be overlooked that particular institutes are in fact in the lead internationally.

To overcome at least the financial stagnation of research, Professor Maier-Leibnitz called upon the Ministers of Culture and Science in the Federal Republic and its component States to increase the capacity for work in the country's universities, by promoting and supporting important research and at the same time making use of existing but insufficiently used research facilities. In this way, with less resources, a considerable improvement in quality could be achieved—something which had been greatly neglected during the continuous expansion of higher education. An increase

Photo: P/B, Bonn

Students at Saarland University: for them, what sort of future?

in the budget of 10% (DM60 million) would be sufficient to begin with, which amounts to only $\frac{1}{2}\%$ of the DM13,000 million which is spent annually on higher education in the Federal Republic.

In this connection it must soon be clarified whether the balance between applied research and pure research is to be maintained in Germany. The former has expanded rapidly over the past few years through government grants. Those private institutions entrusted with the furtherance of science have so far refrained from open criticism of this development. To understand the problem it is only necessary to consider the vast sums which have been spent on applied research. Whereas the state granted DM850 million in 1974 to further general research in universities and private institutions, it allocated DM3,200 million for advanced programmes of applied technology (nuclear research and technology, space research and technology, data processing, marine research and other technology).

Criticism of the disproportion between applied research and pure research may be justified when it is

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considered that Germany is living on the results of past research, without worrying about the future. No politician seems to give serious consideration to the scientific source for the technology of the 1980s. This behaviour is reminiscent of the carelessness with which people treated questions of raw materials and the environment until recently. Many scientists are worried about the situation: it puts the Federal Republic at a disadvantage internationally; it also threatens those industrial nations which are short of raw materials, whose economies depend on the progress achieved in their laboratories.

The final decision on the relationship of pure research to applied research lies with the political authorities. They have never shown any special enthusiasm for pure research, which has little propaganda value for them in comparison with basic technological projects. Publicity and politics are not, as is often claimed, the enemies of science; they are simply indifferent to pure research. The "Gray Paper" ought to bring about a change of attitude. But will any of the politicians read it? □

The state of health of NIH

Colin Norman in Washington looks at the National Institutes of Health, just investigated by a Presidential Commission

ON various occasions in the past few years, the National Institutes of Health (NIH), one of the largest and most prestigious biomedical research centres in the world, has been beset with personnel and budgetary upheavals. Two NIH directors have been fired, ostensibly for political reasons, repeated clashes between Congress and the Executive branch over the overall health budget have frequently thrown

planning at NIH into utter confusion, and many of the agency's programmes have been suffering from chronic shortages of cash while others have been receiving relatively generous support. It has not been a very happy place.

The problems which have plagued NIH and, by extension, the universities and medical schools whose work NIH supports, stem from a variety of

sources. They range from the fact that NIH's budget has climbed to more than \$2,000 million a year, which makes the agency politically very visible, to false public hopes that medical science can produce swift solutions to complex health problems. But the chief irritant is clearly the 1971 National Cancer Act.

The instrument which officially launched the federal war on cancer, the act abruptly changed many established methods of supporting, planning and managing biomedical research. It also elevated the National Cancer Institute (NCI) to a privileged position in NIH, and caused vast sums of

money to be poured into cancer research while other areas of biomedical research have been growing slowly, if at all. But the Cancer Act, more than any other single factor, has sharply focused political attention on NIH's affairs.

Early in 1974 when morale at NIH was at a low ebb, following some particularly severe upheavals, Congress, at the instigation of Senator Edward M. Kennedy and ultimately with the support of the then Secretary of Health, Education and Welfare, Caspar Weinberger, passed a bill establishing a Presidential Commission to investigate NIH's affairs and make recommendations for relieving some of the strains. Chaired by Franklin D. Murphy, a former medical school dean who is now chairman of the Times Mirror Corporation in Los Angeles, the panel conducted an exhaustive 15-month inquiry, taking evidence from scores of witnesses and commissioning several independent studies of key aspects of federal health policy. It submitted its long-awaited report to President Ford and Congress on April 30.

A plea for more stability in funding, and for NIH to be left under the control of scientists rather than politicians, the report with its voluminous appendices offers few radical suggestions for changing NIH's operations. Its central message is summed up in the introduction: "The United States can take pride in a remarkably productive biomedical and behavioural research effort. The panel is convinced that despite the appearance of strains in the structure and some dislocation in the parts, the edifice is sound".

Though some people have been quick to criticise the report as being a self-serving document (five of the seven panel members were drawn directly from medical schools), it nevertheless offers some constructive analysis of NIH's activities. Perhaps its most far-reaching and controversial suggestion is that a tentative stem should be taken to re-integrate the National Cancer Institute into the rest of NIH. This should be accomplished, the panel suggests, by re-constituting a powerful committee which now oversees the working of the National Cancer Act into a more broadly based body with jurisdiction over the entire NIH effort.

To understand why such a seemingly mild suggestion is controversial, it is necessary to appreciate some of the history of the National Cancer Act and present cancer politics. The act stemmed largely from the recommendations of a blue-ribbon panel of scientists and lay members chaired by Benno C. Schmidt, a New York industrialist with influential political connections and a persuasive manner. The panel, which reported in December

1970, argued that NCI should be separated from the NIH bureaucracy, be given a separate budget and that its director should be appointed directly by the President. An independent status, the panel argued, would give cancer research greater visibility and enable NCI to adopt a more sharply focused research effort.

The plan was fought at the time by many scientists who were alarmed at the prospect of separating cancer research from closely related fields of study, and when Congress passed the National Cancer Act, it kept NCI within NIH, but only just. The NCI director is appointed directly by the President, and the NCI budget is drawn up and submitted separately from the budgets of other NHI institutes. The Act also established a powerful, three-member President's Cancer Panel to oversee the working of the act and to provide an extra link between NCI and the White House. Benno Schmidt was appointed chairman of the Cancer Panel. He has since devoted much of his time and considerable talents to his duties.

Since passage of the Act, the NCI budget has grown swiftly, though not as rapidly as the Act envisaged. Between 1970 and 1975 NCI's budget rose by 280%. In the same period, the budget for the National Heart and Lung Institute doubled (research on cardiovascular diseases was given a boost by a bill passed by Congress in 1972), but the budgets for all the other eleven institutes combined rose only by 20%. Cancer's share of the total NIH budget climbed from 16.6% to about 35%. At the same time, NCI began funding a growing share of its research activities by contracts rather than

grants, a move which created some discontent in the biomedical research community.

Considerable resentment has been expressed at the privileged position of NCI, and the fact that the growth in support of cancer research has seemingly been made at the expense of other less politically favoured areas of research. Nevertheless, the cancer budget has considerable popular support and is defended by a powerful coalition of politicians, cancer researchers and others, not least of whom is Benno Schmidt. It would be politically difficult simply to merge NCI back into NIH, which is why the panel is very cautious in its recommendation.

It begins by noting that it "both recognises and supports the priority for cancer research established by the Congress", and suggests that the special status of NCI be retained "at this time". It recommends, however, that the President's Cancer Panel be reconstituted by statute as the President's Biomedical Research Panel, with responsibilities extending over all functions of NIH. Its membership should be broadened, it should provide a link between NIH and the White House, and offer advice on the total biomedical research effort. Finally, the panel suggests that "this proposal provides the opportunity, if experience so dictates, to fully integrate the national cancer programme with the other programmes of NIH in due time". The panel's report notes that Benno Schmidt, who was one of the seven panel members—further testimony to his influence over biomedical research matters—did not participate in decisions leading to this recommendation.

Panel proposals

THE following are among the more important recommendations of the President's panel.

- The President's Cancer Panel should be reconstituted and its responsibilities should extend over all NIH programmes.
- A new NIH advisory board should be established to provide advice to the Director of NIH and a measure of public input into NIH policy.
- No new research institutes should be established, and consideration should be given to amalgamating some existing institutes.
- The research programmes of the Alcohol, Drug Abuse and Mental Health Administration should be strengthened, but the panel decided, by a 4 to 3 vote, to recommend against transferring them to NIH.
- Investigator-initiated grants, rather than contracts, should continue to be the chief means of supporting

research by NIH.

- The Director of NIH should have at his disposal a contingency fund of not more than 1% of NIH's budget, to initiate new studies.
 - Legislation should be passed to preserve the secrecy of peer review deliberations and to protect the confidentiality of grant proposals until they have been funded.
 - NIH should resist pressures to participate in long-term patient care.
- The panel members were: Franklin D. Murphy, Times Mirror Corporation (Chairman); Ewald W. Busse, Duke University Medical Center; Robert H. Ebert, Harvard Medical School; Albert L. Lehninger, Johns Hopkins University Medical School; Paul A. Marks, Columbia University College of Physicians and Surgeons; Benno C. Schmidt, J. H. Whitney and Co.; and David B. Skinner, University of Chicago Hospitals and Clinics.

The proposal is likely to be controversial for several reasons. First, many scientists who have been resentful of the privileged position and burgeoning budget of the cancer programme will not be happy to see the panel responsible for much of that growth placed in charge of the rest of NIH. In that regard, however, it should be noted that while Schmidt has skilfully fought for the interests of the cancer programme, he has also spoken out on many occasions against letting NCI's budget grow at the expense of other areas of NIH. A second factor is, however, potentially more disturbing. The establishment of a powerful committee to oversee the programmes of NIH, though it will have no direct executive power, could seriously undermine the authority of the NIH Director. Though the present incumbent, Dr Donald S. Fredrickson, said in an interview last week that he would willingly work with such a panel if it is established, he would clearly not relish the thought of there being two Popes in charge of NIH and finding that he is the one in Avignon.

Another major point which the panel

addresses is the confusion introduced into NIH planning by the repeated scraps between Congress and the Administration over the budget for the Department of Health, Education and Welfare, of which NIH is a part. Every year, Congress approves a larger budget for HEW than the president requests, and the matter becomes a political football with vetoes, budget deferrals and so on clouding the picture. Consequently, NIH frequently doesn't receive its budget until the fiscal year is nearly over, and it doesn't know until the last moment how much money is going to be available.

Fredrickson acknowledged last week that such uncertainties are a "very serious problem for NIH". But it is difficult to see a solution. Certainly, the panel's recommendations are unlikely to be accepted. The panel, in short, suggests that Congress and the Executive Branch should approve a new budgeting system for NIH which would allow forward funding of multi-year grants and contracts through a single appropriation instead of the present system under which new budgets must be approved each year. That

system would, however, introduce more inflexibility into the federal budget, and would thus run counter to the desires of this Administration. The proposal, moreover, could just as easily apply to the rest of the Federal Government's \$20,000 million-a-year research budget, so the Administration would be wary of setting a precedent.

Finally, it should be noted that the strains within NIH and the rest of the biomedical research community have been less apparent in recent months. In part, that is due to the introduction of some new policies, such as a new granting arrangement in NCI to replace some contracts, and improvements in the quality of peer review of contract proposals. It is also due in part to the appointment last year of Fredrickson as NIH Director. A skilled administrator who has earned the respect of both scientists and politicians, Fredrickson has had a considerable calming influence. Nevertheless, many of the fundamental problems which have led to discontent in the past remain, and it is unlikely that the panel's pitch for more independence for NIH will solve them. □

USA

Human rights guidelines adopted

Colin Norman reports on moves in the United States to combat the repression of scientists

FOLLOWING a recent spate of complaints that it has been too timid in speaking out against violations of human rights, particularly those of scientists, the National Academy of Sciences has adopted a set of guidelines to govern its actions when future infringements are brought to its attention. One of the Academy's most outspoken critics on such matters, Dr Jeremy J. Stone, Director of the Federation of American Scientists, last week welcomed the guidelines as offering a "constructive approach".

Proceeding from the observation that "violations of human rights . . . occur in many—perhaps all—countries", the guidelines suggest that the Academy should try to battle such violations by "persuasion on moral humanistic grounds". But they add that this approach "may require fortification by stronger measures".

The Academy operates for the US government a number of scientific exchange agreements with other countries, which gives Academy officials considerable contact with their foreign counterparts. In the past, the Academy has used such contacts to make private, face-to-face representations on behalf of beleaguered

scientists, particularly repressed Soviet intellectuals. But only in the case of Andrei Sakharov has the Academy issued a public protest, and a number of scientists, both inside and outside the Academy, have recently criticised it for not speaking out more forcefully on other occasions.

The guidelines state that the Academy will continue to remonstrate influential officials in other countries, choosing individual cases of scientists and engineers to protest. "We will continue to use the quiet informal contact as our principal mode of communication with peer groups and governments in other countries", the guidelines state, but they add that "we do not eschew entreaty by public vehicles; indeed, we anticipate that such action will occasionally be appropriate". The strongest leverage which the Academy has in such negotiations is the threat of withdrawal from participation in exchange agreements, and the guidelines recognise that such a measure is available. Indeed, "it is always implicit in the background, but (it is a measure) that we can use only rarely".

In a related development, Academy members adopted a resolution at the annual meeting last month affirming principles of freedom of inquiry and expression. They urged colleagues around the world to join them in

sending the following (or similar) signed statement to the Academy:

I hereby affirm my dedication to the following principles.

- That the search for knowledge and understanding of the physical universe and of the living things that inhabit it should be conducted under conditions of intellectual freedom, without religious, political or ideological restrictions.
- That all discoveries and ideas should be disseminated and may be challenged without restriction.
- That freedom of inquiry and dissemination of ideas require that those so engaged be free to search where their inquiry leads, free to travel and free to publish their findings without political censorship and without fear of retribution in consequence of unpopularity of their conclusions. Those who challenge existing theory must be protected from retaliatory reactions.
- That freedom of inquiry and expression is fostered by personal freedom of those who inquire and challenge, seek and discover.
- That the preservation and extension of personal freedom are dependent on all of us, individually and collectively, supporting and working for application of the principles enunciated in the United Nations Universal Declaration of Human Rights and upholding a universal belief in the worth and dignity of each human being."

An Academy official said last week that the Academy will act as repository for such statements and make public the response. Confidentiality will be guaranteed to those who request it.

Signed statements should be sent to the National Academy of Sciences, 2101 Constitution Avenue NW, Washington DC 20418. □

COMECON

As a result of the recent meeting of the Comecon Executive in Moscow, coordination of economic plans will provide the chief means of cooperation for the Comecon countries between now and 1980. A programme for this integration produced last June includes specialisation and co-operation on key scientific and technological problems, and a strengthening in the energy, fuel and raw materials sectors.

One of the main fields of cooperation is atomic power engineering. According to the Joint Forecast for the Development of Nuclear Power Engineering in Comecon for the period up to 1990, the combined capacity of atomic power plants in the Comecon countries by 1980 will be some 30 million kW. As with most Comecon projects, the Soviet Union plays the leading role in such developments, but the contribution of the other member countries to joint research and the exchange of nuclear technology is stated to be "constantly increasing". In 1975 this mutual exchange is said to have increased by 23%. Though no precise basis for this figure is specified, an increasing amount of work in this field is apparently being carried out beyond the Soviet frontiers.

Thus a joint investigation into nuclear breeder reactors by the Institute of Nuclear Research (Swierk, Poland) and the Institute of Nuclear Power of the Byelorussian Academy of Sciences is being carried out at Swierk, using the *Maria* reactor. Some bipartite agreements in this field have also been signed between individual member countries. These include a programme of co-operation between the Czechoslovak and Hungarian Atomic Energy Commissions covering reactor physics, the development of nuclear instruments and the development of nuclear power stations. Talks between J. Neumann and Gyocrgy Osztrovszki, the Commission Chairmen, included discussion of the Heller-Forgo system of air-condenser cooling for reactors. Similar bilateral discussions have also been held between Bulgaria and Rumania.

The Conference of Comecon nuclear power engineers, which met earlier this year in Rheinsberg, East Germany, made no absolute claims for future expansion: the share of nuclear energy in the growth of electrical energy in the Comecon bloc over the last five years was 17%, it was said, and this "should" increase to 27% in the next five years. The general picture, however, is one of optimism. Poland is introducing a

special training scheme for nuclear engineers at the Cracow Academy of Mining and Metallurgy, using a 100 kW nuclear reactor provided by Swierk, and the Soviet Union is expanding the field of cooperation outside the Comecon block, assisting the Finns in the construction of a nuclear power station at Loviisa.



Cancer research provides another major field of cooperation. A conference in March of this year, held at the National Institute of Oncology in Budapest, laid down guidelines for future cooperation and integration. Hungary is to be in charge of pharmaceutical research and the testing of drugs, and the Soviet Union, notably the Moscow Oncological Institute, is to continue its research into the feasibility of neutron therapy, in which it is claimed that "substantial progress" has already been made. A Comecon X-ray morphological and diagnostic laboratory has been opened in Obninsk, and the possibilities of using π -mesons and proton radiation for the treatment of carcinomas is under consideration. This line of research, it is postulated, could be carried out with the cooperation of the Dubna Nuclear Research Institute.

The conference considered it particularly important to extend its contacts with the international cancer research organisations. WHO, the International Oncological Union, and the European Cancer Research Organisation already have links with certain Comecon States; the new proposals, however, suggest information-sharing between these bodies and the Comecon programme as a whole, presumably through the Moscow Oncological Institute, which acts as a co-ordinating centre and clearing-house for Comecon cancer research.

● One promising outcome of the recent agricultural crises in the Comecon block is the availability to

the USA of credits from the sale of "agricultural surpluses" to Comecon countries. In the case of Poland, some of these credits are to be used to establish the "Maria Skłodowska-Curie" fund to finance joint research, the exchange of specialists and the organisation of conferences, thereby providing a fresh stimulus to Polish-US scientific contacts. Recent joint undertakings between the two countries include a joint expedition to the North-West Atlantic to study plankton resources, and hence the possibility of developing new fishing grounds in the area.

● At a meeting in Luxembourg in February of this year, Gerhard Weiss, the Chairman of the Executive Committee of Comecon, and Gaston Thorn, the President of the Council of (Prime) Ministers of the European Community, discussed a number of fields of cooperation between Comecon and the EEC, including standardisation, conservation, and statistics. Regarding conservation, the Comecon countries and Yugoslavia have now affirmed their willingness to see closer cooperation with the EEC. This emerged at a symposium in Dresden on technical questions relating to production processes with little or no waste products. Dr Hans Reichelt, the East German Minister for Environmental Protection and Hydroeconomy, welcomed Mr Brezhnev's proposal that an all-European Congress on cooperation in environmental protection should be held in the near future.

● The Royal College of Psychiatrists in the UK has adopted a resolution reiterating its condemnation of the abuse of psychiatry for political purposes in the USSR, and has pledged itself to do all in its power to secure the passing of a similar resolution by the World Psychiatric Association at its next World Congress in Honolulu in 1977. The proposed resolution runs counter to the expressed opinion of Dr Denis Leigh, the Secretary General of the WPA, who would prefer that all resolutions should deal with general ethical principles and avoid questions connected with religion, national policies, political belief and so on. He told the English-language journal *Soviet News* in December that he felt it important that the Soviet Union should be represented at the Honolulu Congress, and described the Soviet Society of Neuropathologists and Psychiatrists as "one of the most loyal" members of the World Psychiatric Association.

Vera Rich

SWEDEN

Science from the top

Wendy Barnaby reports from Stockholm on the International Foundation for Science's aid programme.

FEW activities can cause such disillusion as the giving of foreign aid. Programmes hailed initially as life-savers often peter out with the sponsor complaining of lack of cooperation by the receiver, who in turn accuses the sponsor of paternalism or outright exploitation. Often the least satisfactory outcomes seem to bedevil the wealthiest sponsors. Money means organisation and, *ipso facto*, the diversion of creativity away from aid-giving into the maintenance of the organisation itself.

If wealth tends to bring problems, the International Foundation for Science (IFS) should on the face of it have more success than most aid organisations. Its total revenue for 1976 is expected to be Sk3,560,000 (about \$790,000). The organisational side of the IFS looks healthy, too: it was formally established at a meeting in Stockholm last September as an afterthought to the initiation of some 45 projects. The only full-time employees are five administrators.

Each project is actually a research grant given to a young scientist from an underdeveloped country on the condition that his or her work is relevant to the needs of the country and carried out in it or some other underdeveloped land. The grants are given annually and are renewable for four years. The original intention behind the scheme was to try to stop the brain-drain of young African, Asian and Latin American scientists to developed countries because of the paucity of equipment and contacts at home. The grants—there are now 100 of them—have all so far been given within the broad field of biology applied to food production.

This encompasses six specific areas. The first is the development of local fish-farming through research on fish, of which an example is a grant of \$13,000 given to a project in Nigeria's Federal Department of Fisheries to study the distribution of the popular cat-fish in order to collect fingerlings for stocking ponds. The second area is the improvement of small animals (mainly goats and sheep) of domestic and local importance—for example the research being done at the National Agricultural University in Lima to use agricultural waste products as more efficient food for guinea pigs, which are

the main source of animal protein for the poor population in the upland region of Peru. The IFS has contributed \$4,600 to the project. It also supports work on the morphology, physiology and pathology of vegetables, tubers, pulses and forage crops, such as that at the University of Malaya in Kuala Lumpur, to study the protein values of locally grown leaves, stems and tubers from cassava. This study—to which the IFS has given \$2,300—is aimed at improving cassava for direct human consumption or for the production of leaf protein concentrate.

Studies of mycorrhiza (the symbiotic association of a fungus with the roots of a seed plant) in connection with afforestation form the fourth area of interest. At the Forest Products Research Institute, Kumasi, Ghana, research is being done into the most suitable fungi and the performance of field inoculation of pines with mycorrhizal fungi, the mass production of suitable fungi and the performance of the inoculated pines. The results will, it is hoped, contribute to Ghana's re-afforestation programme, and the IFS has supported it with \$13,000. The fifth area is the improvement of traditional food fermentation processes—for example, a project under way at the University of Ain Shams in Cairo, Egypt, in which the waste materials from industrial food processing are being examined to see if they are suitable for controlled microbial growth which could lead to the economic production of protein. The IFS has given the project \$7,000. The investigation of organic compounds from natural products traditionally believed to have medicinal applications is the sixth area. Here, the IFS has for example given \$6,300 to research at the Haile Selassie I University, in Addis Ababa, Ethiopia, into the structure, properties and possible toxicological effects of a local plant used against tape-worm infections.

The fact that the grants are given in so many different places is not the only international aspect of the IFS. The funds are also contributed from many countries, mainly through national academies or other bodies representative of natural or social science or technology. In 1976, Sweden is expected to contribute just over half the total budget, Canada nearly one-fifth, and France, Belgium, the Federal Republic of Germany, the Netherlands and Japan will make up the rest between them. The President of the organisation—and the moving force

behind its inception—is Swedish Professor Sven Brohult.

The specific projects mentioned so far illustrate the IFS pattern of a small grant being given to an individual scientist already employed at a research institute in an underdeveloped country. The organisation's involvement does not end there, however. Applications for grants in each of the six areas of interest are perused by scientific advisory groups, who recommend on their adoption to the Grants Committee. The advisers (who work for the IFS voluntarily) are scientists employed on research within the area whose applications they judge, and travelling in the course of their work to the regions in which projects are being carried out. In this fashion, the IFS is building up a network of contacts between researchers working in the same field in neighbouring countries who were not previously aware of each other's existence.

The IFS is so far committed to the potential of developed countries' science for aiding underdeveloped countries, a commitment which grew naturally out of its attempt to stop the brain-drain and one which can be strongly defended given the fact that so many researchers from underdeveloped countries have already been educated abroad. With this commitment goes a "science from the top" approach: support of researchers educated in science working by traditional methods in the hope that their work will ultimately benefit the other sectors of society. This is less fashionable now than the "science from the bottom" approach: educating the masses to contribute to the improvement of their condition through science, as for example Chinese peasants reporting on signs they see in the fields of approaching earthquakes. Ideally, each approach should complement the other. In the meantime, however, the IFS is seen to be doing something worthwhile. □



Sven Brohult, IFS President, and Olle Edquist, former Executive Secretary

IN BRIEF

Sea conference ends

The latest eight-week session of the 156-nation Law of the Sea Conference ended in New York last Friday with several bitterly-debated issues still outstanding. When the next round of talks is convened at the same venue in August, subjects for further discussion will include the extent of national jurisdiction and sovereignty over economic zones, the rights of land-locked states, the most suitable form of disputes procedure, and freedom of access for scientific research—a particularly contentious area offering scope for espionage and mineral plunder, in the view of some third world nations. Nonetheless, the divergent views of the underdeveloped and industrial states have closed slightly at least on the issue of control over deep sea mineral exploitation, and a compromise solution seems within reach. As the conference ended the Soviet Union pro-

tested at recent US legislation to extend its fishing limits to 200 miles by March 1977. One view, however, is that the US move would hasten that similar unilateral action which other worried states would probably have taken anyway.

Fluorocarbon report delayed

A crucial report by the National Academy of Sciences on the possible effects of fluorocarbons on the ozone layer has been delayed by up to three months because of new data which suggests that the threat may not be as serious as first predicted. It seems that chlorine, liberated from fluorocarbons, in the upper atmosphere, may react with nitrates to form a moderately stable compound. If correct, the theory, which is backed up by some direct measurements, means that the reaction would provide a "sink" for destructive chlorine atoms, reducing their effect on the ozone layer substan-

tially. The Academy report, which was in the final stages of drafting when the new data came in, has been delayed until July.

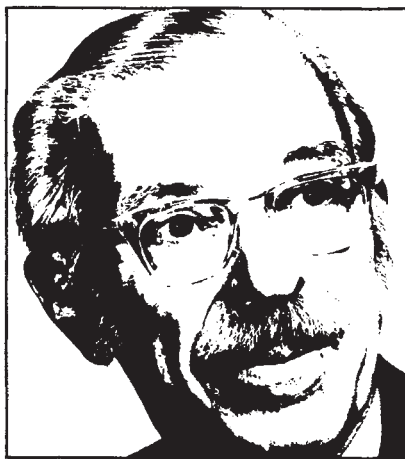
Monitoring the stratosphere

Spurred by the recommendations contained in February's US Government decision granting Concorde commercial access to the United States for a 16-month trial period, Britain and France have entered a joint programme with the US to monitor the stratosphere. Under the agreement, signed in Paris last week, the participants will aim to improve and extend monitoring techniques, and record long-term fluctuations in global ozone levels. The US recommendations followed concern that nitrogen oxides in supersonic aircraft exhaust might destroy stratospheric ozone through reactions like those involving fluorocarbons from aerosol sprays.

SCIENTISTS have laboured long and mightily in the past half-century to help farmers increase food production, and to identify and prevent nutritional deficiency diseases. Their efforts were so successful that a surplus of food piled up in the USA, although not in most other countries, and there was a great deal of overeating. Simultaneously, many infectious diseases ceased to be significant causes of mortality, as a result of public health measures, including immunisation, and the discovery of chemotherapeutic drugs and antibiotics. Deaths from cardiovascular disease, cancer, hypertension and other degenerative diseases went to the head of the statistical tables. It is an obvious and easy oversimplification to blame these on food because of their association with obesity, especially the cardiovascular group. But the fact that overeating leads to fatness is no reason for blaming the food rather than the gormandiser.

When the choice between guns and butter was rhetorically offered by Goebbels to the German public, little did we expect that, a few years later, butter would be placed with guns among the enemies of human beings. Yet *Nature* (April 15, page 565) wants every cow to carry a government health warning for making butterfat. The warning should be on the bathroom scales, not on the cow. The warning would be just as logically placed on every nubile woman, for human milk contains fats similar to those in cow's milk, and we are told that pre-arteriosclerotic changes can start at a very young age. I doubt that this suggestion will get very far.

In fact, when I was asked recently what foods were not under suspicion of being unsafe to eat, I could think of only one: sawdust. Fats, of course, are deadly; the saturated kind cause

Mutiny on the Bounty**THOMAS H. JUKES**

heart disease and cancer, and the unsaturated ones destroy vitamin E. Sucrose is belaboured as being addictive and diabetogenic, except, apparently, when it occurs in orange juice (5% sucrose) and pineapple juice (8% sucrose), or when it is brown instead of white. School children who eat candy bars are being reprimanded by adults who smoke cigarettes and drink vodka on the rocks. School authorities are shutting down the vending machines, so that enterprising pupils are bootlegging soft drinks.

We eat too much protein, of

course, about twice as much as we need, and we had better feel as guilty as possible about this. The US House of Representatives just passed a bill to prevent the Food and Drug Administration from restraining the promotion of para amino benzoic acid as a vitamin, much to the joy of the "health food" stores. For if food makes you sick, vitamin pills must make you well, even non-vitamin pills that are only good for bacteria.

Food additives must go, say the consumerists, and the large companies follow suit by devising all-natural breakfast foods; nothing artificial added, no preservatives. Why let the big and growing market for "natural foods" go elsewhere? Sodium propionate stops bread from going mouldy, but it is artificial, even though it is normally present in cheese, so out it goes. Never mind the fact that some moulds produce carcinogens. Bacon is the villain, it contains nitrites, which form nitrosamines. However, more than two-thirds of the nitrites entering the average stomach come from saliva, and 86% of the nitrates, from which nitrates are formed in the mouth, come from vegetables.

Sawdust and bran alone remain, because we need more fibre to keep the bowels active, and thus prevent cancer. This is proved by the fact that there is very little cancer in Africa, where there is lots of movement. Oh, well! The English have always been preoccupied with that function, ever since the days of Beecham's Pills and Kruschen Salts.

Maybe we were better off when we counted nickels instead of calories.

correspondence

International exchange

SIR,—The fourth European *Drosophila* Research Conference met in Umeå Sweden, in the Spring of 1974. It was a very interesting conference, which was attended by more than 150 *Drosophilists* from all over Europe. However, a certain disappointment was apparent among many of my colleagues, because no *Drosophilists* from the Soviet Union attended.

The fifth Conference will be held in Louvain-La-Neuve, Belgium, at the beginning of next September. As the organiser of the conference I really hoped that, as well as the other European *Drosophila* geneticists, a delegation from the USSR could attend the conference. Therefore, at the suggestion of an American colleague, I wrote last July to Academician Belyaev, who is President of the Soviet Society of Genetics, in order to obtain from him a list of his colleagues who might wish to attend the conference. I received a list of about 20 names last September.

At the beginning of January, I sent a first announcement and preliminary registration forms to all of them. At the same time I wrote to the Chairman of the USSR Academy of Sciences, inviting officially a delegation from the USSR to attend the conference. I got some answers from the *Drosophilists* of the USSR who were eager to attend.

Recently, however, I received a very short letter from the foreign relations department of the Academy telling me that the scientists of the Academy do not plan to attend the conference. It is hard to believe that it constitutes a final answer, especially as the XIV International Congress of Genetics is supposed to be held in the USSR in 1978. Are we to understand that the exchanges between geneticists from West and East Europe are only one way?

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Birds on the Chagos Bank

SIR,—The recent communication from Hiron *et al.* (April 1, page 387) prompts this response. Their suggestions relative to rats and birds at Great Chagos Bank raise several questions. "Feral brown rats" (are those *Rattus norvegicus*?) are alleged to prevent full utilization of Eagle and Egmont islets by seabirds. The suggestion is made

that "warfarin" and Liverpool virus" be utilized to remove the rats.

While many studies of island situations have indicated the predatory impact of rats on nesting seabirds, this is not a universal relationship. Our own studies at Enewetak Atoll in the Marshall Islands have failed to produce evidence of predation by rats (*R. rattus* and *R. exulans*) on such colonies, which include one of more than 10,000 sooty terns.

Eradication of rats is difficult. If the rat colony is at all sizeable, the presence of the "warfarin" resistant gene is likely; and problems might well be encountered. I wonder whether the Liverpool virus is being confused with Salmonella bacteria, a now discounted mode of control. In this connection, the WHO caution against their use should be noted (FAO/WHO Expert Committee on Zoonoses, *Wld Hlth Org. techn. Rep. Ser.*, No. 378, 1967; WHO Scientific Group, Ecology and Control of Rodents of Public Health Importance, *Wld Hlth Org. techn. Rep. Ser.*, No. 553, 1974).

This suggests that the terrestrial ecology of those islets should be adequately understood before drastic management efforts are attempted. Knowing more of the "recent surveys" would be most helpful.

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Cows and heart disease

SIR,—If a non-medico may presume to comment on coronary heart disease (Editorial, April 15), surely it is not the sacred cow that needs to carry a government health warning, but the golden car. Ignoring the nauseating exhaust products of the latter, I refer to the long term debilitating influence of car commuting. And not merely to confined physical inertia, but to the tremendous stresses involved in co-existence with one's neighbour within the relentless daily stream of traffic. To have a cyclist's eye-view in Boston, for example, is to see men possessed to a degree dangerously approaching that of mechanised Gadarene swine.

You say "Strangely, there is no evidence of a similar trend [of increasing incidence of CHD] for women". Is this because Equal Opportunity has not yet advanced far enough to take women from the physical toil of house-keeping and put them on equal wheel-

ing with male commuters? But for the normal (or not so normal these days!) healthy body, let taste, not diet, remain the final arbiter. No doubt those in bondage to the golden car will regard this as mere folly and scandal, yet that symbol of supposed "freedom" increasingly becomes the premature reaper, whether we are saturated or unsaturated.

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Isolative sound-change

SIR,—I should like to propose the following explanation for the isolative vowel sound-change from Anglo-Saxon *ā* through Middle English *ȣ* to Modern English *ō* (as in *more*) instanced by Alan Ross (April 22, page 664).

If you open your mouth, say "ah", and continue the utterance while gradually allowing your mouth to relax and close you will find yourself recapitulating, in the course of seconds, the same sequence of sound changes as your correspondent cites as having occurred over centuries, and for the same basic reason, namely a gradual tendency to open the mouth less wide to make the vowel sound—in a word, laziness. (It is interesting to compare the current marked tendency for the modern Persian *ā*—which is nearer to "aw" than to "ah"—to lapse into *ū*, for example, *Teherān* to *Teherūn*).

In some areas of England, where vowels are stronger, the transition has not yet reached the third stage. To give a ready example, listeners to the BBC television series "When the Boat Comes in" may have noticed that the pronunciation there of *boat* still approximates to the Middle English *bȣt*—as its surviving spelling indeed suggests it should.

I believe that the laziness principle also explains the tendency to lapse from *fire* to *far*, and so on. And surely it accounts for the tendency for our short vowels—*a* as in *Indian*, *e* as in *absent*, *i* along with *o* as in *station*, and *o* as in *onion*—to lapse into the basic *un*-sound found onomatopoeically in *grunt*. But I would not suggest that it could account for all isolative vowel changes, because undoubtedly there is a complex of physical and psychological factors involved.

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news and views

CO₂ variations and the Southern Oscillation

from John Gribbin

THE so-called "quasi-biennial" oscillations of the atmospheric and oceanic system, which produce variations in weather parameters with 'periods' of anything from a year to at least half-a-dozen years, but usually averaging between two and three years, are among the best known and most intensively studied climatic phenomena—but not necessarily the best understood. One very important member of this family is the Southern Oscillation, which is most clearly identified by variations in pressure with a timescale of two to two and a half years over the Southern Hemisphere; these variations are often very highly correlated between the two key areas around Jakarta (Indonesian low) and Easter Island (South Pacific high), and to a lesser extent the variations can be correlated with features of the Northern Hemisphere circulation. The Southern Oscillation is not, then, some local or even hemispheric phenomenon, but rather a particularly obvious manifestation of the general tendency of the atmosphere/ocean system to vary on this kind of timescale, and study of just how this oscillation does interact with other parts of the atmosphere/ocean system is important in the context of understanding quasi-biennial oscillations in general—and, ultimately, producing better forecasts.

Lamb has succinctly summed up the situation (*Climate: Present, Past and Future*, 1, Methuen, 1972): "the Southern Oscillation and its roughly 2- to 3-year periodicity can clearly be taken to involve an alternation in the pattern and rate of heat distribution affecting much of the world and its wind circulation . . . the Southern Oscillation should be seen as related to the liability of the equatorial Pacific to some of the biggest and most extensive anomalies of sea surface temperature in the world". These anomalies have one very obvious and significant effect in the phenomenon known as El Niño, which brings high rainfall to generally arid regions of the equatorial Pacific, and although the link between this phenomenon and the Southern Oscillation is not entirely clear, anomalous conditions at the Easter Island high do seem to be involved.

It is with the importance of an understanding of the Southern Oscilla-

tion to our understanding of the whole atmosphere/ocean system in mind that the contribution by Bacastow on page 116 of this issue of *Nature* should be read. Bacastow claims that after a great deal of filtering to remove both seasonal effects and the long-term increase attributed to man's activities the record of carbon dioxide variations in the atmosphere indicates a correlation with the Southern Oscillation. Critics might take Bacastow to task for making the most of somewhat limited statistical evidence which he admits involves adjustments in some cases so that "the fit appeared reasonable to the eye", and which even then end up with correlations which although high enough to be interesting are not conclusive on the basis of these limited data alone. In addition, the very small variation in carbon dioxide levels (less than one third of 1%) seems to occur before the changes in the Southern Oscillation which might be expected to drive them. Nevertheless, although it seems unreasonable to suggest that the carbon dioxide variations might drive the oscillation, there remains the important possibility that both are being driven, or at least modulated, by some outside influence which has yet to be identified.

The somewhat tentative nature of the model proposed is, however, in-

dicative of the tentative state of our understanding of the way the atmosphere and ocean interact. It is easier to appreciate that a thorough understanding of atmospheric variations can only come hand-in-hand with a thorough understanding of oceanic variations, but much harder to see where to begin to tackle the enormous problems involved in developing such an understanding. If the views of some climatologists are to be taken at face value, there is now also an element of a race against time to determine the natural patterns of such change before they are destroyed by man's activities—the controversy about carbon dioxide levels and man being a case in point—and prediction of these anthropogenic effects can only be achieved after an understanding of the natural processes has been developed. The specific case of the interaction between the Southern Oscillation and carbon dioxide uptake of the southern ocean is of direct relevance to the controversy over carbon dioxide pollution and its effects on the atmosphere and oceans; the broader view—what drives all these quasi-biennial oscillations—is perhaps of even greater importance now that the world food production and distribution systems can be so easily shaken by "bad" weather, as we have seen in recent years. □

More speculation on human evolution

from Robin Weiss

WHILE fossil finds hit the headlines, comparative biochemistry also has its place in the discourse on the origins of our species. Two papers in this issue of *Nature* take up this discussion, one from the viewpoint of amino-acid sequences in proteins, the other of nucleotide sequences in DNA. Romero-Herrera *et al.* (page 162) discuss the amino acids residing at two positions in the highly conservative muscle protein, myoglobin, which accepts oxygen from the blood. On the basis of the amino-acid residues in positions 23 and

110 of the myoglobin chain, these authors draw up 'cladograms' or evolutionary trees that might indicate the relationship of man to the higher apes. The most economical cladogram in the sense of providing the minimum number of point mutations in the gene for myoglobin shows the orang-utan diverging from the other higher apes and man before the gibbon does. The problem with 'economy' of mutation is that it assumes that mutations are always selectively disadvantageous. This may be true for myoglobin, but resi-

dues in several proteins must be analysed before one cladogram can emerge as the clear choice.

Benveniste and Todaro's paper, also in this issue of *Nature* (page 101), concludes that during most of the Pliocene era, man's direct ancestors lived in Asia, or at least not in Africa. Therefore they would argue that the recent fossil finds by Richard Leakey in Kenya and by Johanson and Taieb in Ethiopia (announced at a news conference two months ago, but not yet published) that appear to push African records of *Homo* back into the Pliocene may not be the ancestors of our present day species.

Benveniste and Todaro's conclusions arise from ingenious arguments based on tenuous evidence. They have used the technique of DNA hybridisation by which the melted strands of DNA will reanneal with complementary sequences. When melted DNA from two species is allowed to reanneal only complementary nucleotide sequences will do so, and the degree of mismatching within those sequences can be measured as the depression in melting temperature in the 'hybrid' DNA compared with that of truly homologous DNA. Thus the degree of genetic relatedness of species is determined by the extent of DNA hybridisation, and by the temperature at which 50% of the hybrid becomes dissociated. Benveniste and Todaro's annealing studies of DNA prepared from tissues of humans and apes broadly confirm earlier work, notably of Kohne and his associates at the Carnegie Institute of Washington, that the non-repetitive DNA sequences of man are more closely related to those of the African great apes, the chimpanzee and gorilla, than those of Asian apes. So far so good, in that these sophisticated biophysical studies of genetic material are consistent with, though perhaps less discriminating than, the morphological studies of classical primatologists or the eyes of children visiting the zoo.

Benveniste and Todaro then proceeded, however, to analyse a tiny fraction of DNA representing about 10^{-7} of the non-repeating sequences, and possessing the coding capacity for a small protein at the most. This set of sequences is distantly related to part of a C-type virus endogenous to baboons, that is, a virus whose genes are incorporated into the host DNA and which is therefore inherited as a Mendelian trait. By synthesising a radioactive DNA 'probe' complementary to the genes of the activated baboon virus one can screen for related 'viral' sequences by annealing the probe to DNA prepared from normal tissues of other species of primates, including man. About 9% of the probe anneals to chimpanzee and gorilla DNA but

only 3% to human DNA and to that of Asian species of ape. The extent of annealing is so small and the mismatching so great, that the melting temperature of the hybrid DNA can only be estimated by a crude extrapolation. Benveniste and Todaro do not think that man is more closely related to Asian apes than African ones since the overall cellular DNA hybridisation patterns indicate the converse. Their thesis is that African apes have conserved a larger fragment of DNA related to the baboon virus than have either Asian apes or man; that this conservation of viral DNA sequences results from natural selection due to some factor in the African as opposed to the Asian environment; that this factor is the baboon virus itself (and that of ancestral baboons) transmitted as an infectious agent; and that the conserved DNA sequences somehow confer immunity to infection of the host by the baboon virus. If man was not exposed to the baboon virus during the Pliocene era, there would be no selection to conserve these DNA sequences and they would diverge as fast as 'viral' sequences have done in other non-African Old World primates.

To launch such a complicated hypothesis on the difference between 9% and 3% of grossly mismatched homology of DNA sequences seems far-fetched. But Benveniste and Todaro show that it fits a general pattern in that sequences in Old World monkeys that are more closely related to the baboon virus have also diverged at a much higher rate among Asian species than among African species. Moreover the evolutionary arguments do not seem to me implausible, indeed they are familiar. There is some evidence in other species (chickens, for example) that endogenous viral gene expression can confer resistance to re-infection and there is also striking evidence from Benveniste and Todaro's previous studies (*Nature*, 252, 456; 1974) that a C-type virus related to the baboon virus has been transmitted to the genome of the ancestor of the domestic cat (of North African origin) but not to an Asian species of the same genus.

Entertaining as these arguments are, on the basis of the evidence presented here they might have been more wisely restricted to the laboratory coffee room. The argument for man's Asian origin is based on the weak homology of a small fraction of a viral probe. There are no data to determine whether the conserved sequences in different African primates represent the same portion of the probe and indeed the viral sequences 'conserved' by natural selection actually diverge more rapidly than the non-repetitive host DNA as

a whole.

An implicit corollary of Benveniste and Todaro's hypothesis is that man is susceptible to horizontal infection with C-type viruses of the baboon type, and that these viruses could be pathogenic to man. C-type viruses closely related to baboon viruses have been isolated from human leukaemic cells by Gallo and his associates and from fibroblastic cells by Panem and Kirsten. I have argued previously that zoonosis, ancient or modern, may be an important concept in the transmission of pathogenic C-type viruses, and that we should not be too perturbed to find that 'human' isolates resemble animal viruses. Benveniste and Todaro's paper hints that they may now be coming to the same opinion. □



A hundred years ago

AT the Crystal Palace Aquarium the hatching of the spawn of Axolotls has been successfully accomplished. There are three young "broods," and some still unhatched spawn, so that the changes in growth during the first few weeks can be seen all together.

A PLAGUE of Field Voles (*Arvicolica agrestis*) has recently visited some of the pastoral farms of Upper Teviotdale and the adjoining districts, which has led to the appointment of a committee of the Farmers' Club of the Locality for the purpose of estimating the amount of damage done. This was found to be very considerable, the vermin eating the pale and succulent bases of the grass, as well as the young shoots. No great destruction of other vermin has occurred in the district, so it cannot be said that the Voles have become particularly abundant from that cause. Similar plagues have before now occurred in the New Forest.

THE *Geographical Magazine* for May contains an article on the prospects for the Arctic Campaign of 1876. While we must be prepared for the necessity of the ships spending a second winter in the north, the writer thinks that possibly the *Pandora*, which goes out this month for news and letters, may meet them coming out of Smith Sound, "with their work done, their great enterprise completed." There is also an article, with map, on the Island of Socotra, which the British Government have arranged to occupy, and another by M. Venyukof on New Maps of Mongolia.

from *Nature*, 14, 35, 36, May 11; 1876.

Sociobiology: a reply

WE are sad and disturbed at the tone of the article by Robert May (*Nature*, **260**, 390; 1976) on E. O. Wilson's *Sociobiology: The New Synthesis*. A widespread debate, originating in America, is taking place around this book and its adoption as a text for college courses. May's article attempts to sidestep the issues being raised by this debate and to remove the discussion to the rather more traditional and secluded arena of scholarly exchange. The term 'debate' in this context is, we recognise, distasteful to many scientists with a traditional view of science and we use it advisedly. We feel, however, that the effects of science upon everyday thought and upon political doctrines are issues that cannot be resolved by courteous sword-crossing within the language of 'pure' science itself.

On the contrary, the issues raised evoke the deepest feelings about power and its abuse, and about social injustice in ways that inevitably force a confrontation between individuals and groups holding disparate sets of values. We must learn to see what May describes as 'a certain incivility' as a function of the importance of such debates/confrontations. Since consideration of the social bases and the social effects of science should be recognised as being integral to science and its practice, such impassioned and political arguments should appear more regularly in the columns of *Nature* and *Science*.

More importantly, May's focus on the so-called incivilities deflects attention from the serious nature of the issues posed by the publication of Wilson's book. The group of American critics (the Sociobiology Study Group of Boston Science for the People) to whom he refers, see in Wilson, essentially correctly we believe, a prime example of the scientific specialist (the naturalist in the broad classical sense) whose prolonged study of the bee and the ant have fostered in him a rather conservative expectation of the human capacity and potential for change. While such conservatism may well be largely unconscious and not overtly political in expression, untold damage is wreaked on 'informed' people's capacity to think critically about social institutions. By attempting to fit *Homo sapiens* into his evolutionary biological schema Wilson jeopardises any attempt to change present patterns of social inequality. As such it seems to us that 'a certain incivility' is more than reasonable.

Wilson's American critics have raised important objections to his work. They show how his case is based on specula-

tion. He postulates genes, or groups of genes, for such traits as spite, military discipline, blind faith and indoctrination. He uses metaphors from human societies to describe animal societies and in so doing imputes barter, religion and magic to animals. He closes off the theory from tests against the real world by postulating, for example, multiplier and threshold effects which make every observation fit the theory. May attempts to belittle this critique: "To suggest, for example, that the evolutionary considerations which determine the mating systems of mammals and birds have any light to shed on the tensions and asymmetries observed in human sexual relationships is [according to May] to invite reflexive dismissal as a 'sexist'!" Tensions and asymmetries are delicate words indeed to express centuries of economic and social exploitation of women. And in fact, Wilson does try to establish the inevitability of sexism by asserting it has a genetic basis. In an article in the *New York Times* (October 12, 1975) he states concerning the division of labour between the sexes: "This strong bias persists in most agricultural and industrial societies and on that ground alone appears to have a genetic origin . . . My own guess is that the genetic basis is intense enough to cause a substantial division of labour even in the most free and most egalitarian of future societies . . ."

That our society should continue to be so susceptible to such arguments expresses both a past and present deference to expertise in general and a deference of social science to biology in particular. At present we learn in school level biology courses and thereafter the roster of emergent properties that distinguishes life from inorganic matter. Life transcends the purely physical and chemical laws that govern inorganic matter, although matter in motion remains its physical basis. We must ask why, in our society, the human sciences cannot emancipate themselves from biology in the same way that biology is emancipated from the physical sciences. We are unique animals who, while having a physical basis in organic life, transcend it through language and culture.

The final issue is, of course, how does the cultural *status quo* change? It seems obvious to us that social change occurs when humans set about self-consciously to change unacceptable oppressive and outmoded social conditions.

*The Science as Ideology Study Group of the British Society for Social Responsibility in Science**
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*Members include: Anne Cooke, Jonathan Cooke, Mary Fuller, Dorothy Griffiths, Susie Orbach, Joe Schwartz, Bob Young.

ROBERT MAY REPLIES: The above letter makes plain the differences of opinion between its authors and myself as to the place of passion and politics in science. In particular it is true that I represented the controversy over the political significance of *Sociobiology* as only a small (and unfortunate) corner of a large canvas; I think that is the balanced view.

It should be emphasised that, unlike other recent controversies, the dispute over the political implications of *Sociobiology* is not a clash over substantial and well-defined issues. The Science For The People people do not attack Wilson's book but rather their own chimaerical version of it; in Wilson's words they "furnish me with a political attitude I do not have and the book with a general conclusion that is not there". Readers in any doubt on this score should see the recent exchange between the US Science for the People Study Group and Wilson in *BioScience* (**26**, 182-190; 1976) and the impartial news article in *Science* (**191**, 1151-1155; 1976). □

Panacea for injured nerves

from a Correspondent

WHEN a nerve innervating a muscle is cut the effect on the postsynaptic cell is profound. The muscle atrophies, as shown by a loss of mass and of proteins, there is a marked reduction in the cholinesterase activity present in the tissue, and there is a considerable increase in the number of extra-junctional receptor molecules. Since the onset of these effects is related to the length of the nerve stump left associated with the tissue, and since they can be duplicated by blocking release from the nerve terminal, it has been proposed that nerves exert trophic influences on the tissues they innervate (Guth, *Physiol. Rev.*, **48**, 645; 1968). Considerably less attention has been focused on a trophic effect in the opposite direction, from tissue to nerve, although this possibility was first implied 18 years ago (Brown, *J. Physiol., Lond.*, **142**, 7p; 1958). This situation is now being rectified.

Two approaches have been used to study this suggested transfer of information from the post to the presynaptic cells. First, morphological techniques have been used to examine the effects of damaging the postsynaptic cell on presynaptic or synaptic ultrastructure and, second, biochemical correlates to these structural changes have been sought.

The morphological data have now provided unequivocal evidence that damage to a sympathetic ganglion cell, by cutting or ligating its axons, leads to a retraction of the innervating nerve and the insertion of a Schwann cell process between the pre and postsynaptic elements (Matthews and Nelson, *J. Physiol., Lond.*, **245**, 91; 1975; Purves, *J. Physiol., Lond.*, **252**, 429; 1975). The synapse thus becomes ineffective in that there is no transmission through the ganglion. The reversibility of the process depends upon the method of damaging the axons. If the possibility of regeneration and reformation of functional postganglionic synapses is excluded, then functional synapses on the ganglion cells do not reform either, and many cells die (Purves, *op. cit.*). The preganglionic nerves appear ultrastructurally normal although their size may have changed (Matthews and Nelson, *op. cit.*). Similar effects on the innervation of motor neurones were found previously (for example, Blinzinger and Kreutzberg, *Z. Zellforsch.*, **85**, 145; 1968; Cull, *Expl Brain Res.*, **20**, 307; 1974).

If biochemical rather than morphological changes are studied, however, it becomes apparent that the innervating nerves have been altered. For example, Jensen-Holm and Juul (*Acta pharmac. toxicol.*, **28**, 583; 1970), have shown that damage of ganglion cells by guanethidine results in a loss of cholinesterase activity from both the pre and the postsynaptic elements. Black, Hendry and Iversen (*J. Physiol. Lond.*, **221**, 149; 1972) found that destruction of the ganglion cells, using 6-hydroxydopamine or anti-nerve growth factor, prevents the normal development of choline acetyltransferase, the one enzyme known to be unique to the incoming cholinergic nerves and their terminals. In each of these cases it could be argued that the loss of synaptic contact with the ganglion cells removed the source of the information which the innervating nerve required to maintain its biochemical integrity. Similar changes have also been found in motor neurones. In these experiments the effector (muscle) cell has been damaged and the biochemical response to the injury measured in the innervating nerve, but not in those which impinge upon it. It is intriguing that the response of the nerve depends upon the type of damage inflicted on the muscle. For example, atrophy induced by inactivation of the acetylcholine receptors (using an α -neurotoxin) results in a decrease of choline acetyltransferase activity in the nerve terminals and axons. In contrast, no change in the levels of the enzyme was found when muscle atrophy was induced by treatment with botulinum

toxin, a poison whose exclusive action is presumed to be a block of release from the presynaptic nerve terminal (Giacobini-Robecchi, Giacobini, Filogamo and Changeux, *Brain Res.*, **83**, 107; 1975). Tenotomy-induced atrophy also results in a decreased level of choline acetyltransferase activity in nerve endings whereas that provoked by cortisone treatment does not (Diamond, Franklin and Milfoy, *J. Physiol., Lond.*, **236**, 247; 1974). Hypertrophy which resulted from hyperactivity of the muscle did not result in increased levels of the choline acetyltransferase. Thus, it is not whether you injure a muscle or not that is important, or whether you alter its mass, it is the mechanism you use to produce the functional disturbance that counts.

There are also reasons for suggesting that the neurones which synapse on the motor nerves might be altered when these nerves are damaged. Giacobini-Robecchi *et al.* (*op. cit.*) have shown that when the α -toxin, but not the botulinum toxin, is used, there is a significant decrease in the size of the sciatic nerve, its spinal roots and of the corresponding sensory ganglia.

All these results lead to one conclusion. If neurones or innervated cells are damaged in certain ways then this will ultimately modify the neurones which make synaptic contact with them. The question which currently excites a great deal of interest is how, and in what form, is the information transferred from the postsynaptic cell to the presynaptic nerve terminal and even across several synapses and well into the central nervous system?

Only one molecule, nerve growth factor, has so far been implicated in the transfer of this information. Nerve growth factor is a protein which, as its name suggests, is able to stimulate the growth of neurones but its action is limited to those of the noradrenergic and sensory nervous systems. It is also a molecule which is taken up, fairly selectively, by the nerve terminals and, possibly, the cell bodies of the neurones present in these two systems. As well, nerve growth factor is able to prevent some of the biochemical changes which result from postganglionic axotomy of noradrenergic cells (Hendry, *Brain Res.*, **94**, 87; 1975). Interestingly, this protein can also prevent the depression of ganglionic transmission which results from crushing the postganglionic nerves, if it is supplied to the ganglion at the time of nerve injury (Purves and Njå, *Nature*, **260**, 535; 1976). Thus an additional role of nerve growth factor could normally be to maintain the synaptic contacts on a cell and they, in their turn, could then help to maintain certain biochemical parameters of the postsynaptic cells. It is likely that this effect of nerve growth factor is

achieved by its transfer from the nerve terminals to the cell bodies. Since there is no maintenance of synaptic junctions, nor protection from axotomy, unless exogenous nerve growth factor is administered at the time of injury, it is unlikely that normal circulating levels of the protein are sufficient to influence the cell bodies directly. It needs to be concentrated first in the nerve terminals and then carried to the cell body.

Nerve growth factor cannot, however, be a universal panacea to injured nerves and their processes. Motor nerves, which respond to injury much as sympathetic nerves do, seem unable to concentrate and transport nerve growth factor (Stöckel and Thoenen, *Brain Res.*, **85**, 337; 1975). A direct sensory link transmitting this specialised information from the injured muscle to the nerve is also unlikely (Giacobini, Filogamo, Weber, Boquet and Changeux, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1708; 1973) so it is probable that the signal can be transferred directly. The nature of the signals and how they are transferred, are not known, but the identification of these signals must number amongst the most fundamental questions that neuroscientists could wish to answer. □

Radon to predict earthquakes

from Peter J. Smith

AMONG the many potential earthquake predictors investigated in recent years, one of the most ignored, at least in the West, is the apparently premonitory change in the emission of radon. In a brief conference abstract King (*Eos*, **56**, 1019; 1975) recently reported that monitoring of the radon concentration in soil gas was begun in May 1975 at 18 sites along an active 60-km segment of the San Andreas fault in central California and that the first results indicated "spatial and temporal variations which appear to correlate with local seismicity". At the same meeting Teng *et al.* (*Eos*, **56**, 1019; 1975) also reported that a sampling network had been established along the San Andreas fault from Cajon to Gorman in order to measure systematically the radon content of groundwater. By December 1975, however, no definitive results had emerged, although there were "some interesting variations when compared to the local earthquakes".

The latter study in particular was inspired by a long series of groundwater radon measurements made in Russia; and it would seem that until

now Russia and China have been the only two countries to pursue assiduously the possibility of predicting earthquakes by radon emission variation. Following a visit to China in 1974, for example, Press *et al.* (*Eos*, **56**, 838; 1975) concluded that "some of the work in China on radon emanation as a precursory effect is to our knowledge as good as that being done anywhere in the world". Apparently, the waters in at least several tens of wells are being monitored continually (sampling normally once or twice a day) using electrometers which measure the radon-emitted α -particle fluxes.

The result of this activity is that a precursory increase (or, more rarely, a decrease) in the radon content of water has been detected for at least six Chinese earthquakes ranging in magnitude from 4.3 to 7.9. The precursor times ranged from a few days to a few tens of days, and in most cases the radon anomalies were observed within 110 km of the subsequent shock. In the case of the magnitude 4.9 earthquake of June 6, 1974, for example, the radon anomaly, observed at a distance of 49 km, appeared about 15 days before the shock and produced a radon flux 2–3 times that of the normal background.

In view of this success it seems likely that experimental interest in the radon precursor will increase rapidly in the United States and elsewhere in the near future. In the meantime, however, Pierce (*Geophys. Res. Lett.*, **3**, 185; 1976) has pointed to a consequence of variations in radon emission which could well extend observational methods beyond the direct measurement of radon itself. His premise is that an increase in atmospheric radon resulting from the release of radon from the ground should have an effect on atmospheric electricity—in particular on the atmospheric electric field and on the conductivity of the atmosphere. As long as the effect is large enough, therefore, the monitoring of such quantities should reveal the radon precursor indirectly.

To estimate just how large an effect could be expected, Pierce has set up a highly simplified theoretical model of atmospheric electricity which is meant to be "illustrative rather than definitive". The rationale behind this rough approach is that both the physics of the situation and the numerical values involved are so uncertain that a more sophisticated model would hardly be justified. Moreover, in related work, simplified models have turned out to be surprisingly effective. Be that as it may, the model predicts that a precursory increase (of 100%) in radon emission would lead to an increase in fair weather conductivity of about 50% and an average decrease in electric

field of about 30% (20% by day, 40% at night). On this basis, the obvious parameter to measure is conductivity. Unfortunately, however, it is technically much more difficult to measure conductivity than electric field. The field change may be smaller but is easier to record, and 30% might just be detectable.

But only under ideal conditions. A field anomaly of 20–40% represents an absolute change of 20–150 V m⁻¹, and clouds and precipitation can produce changes of several hundred volts per metre, without a hint of radon variation, by generating space charge. Moreover, even in fair weather conditions, such agencies as waterfalls, surf, dust, power lines, aerosols and air pollutants may create space charge and hence perturb the normal field. With problems like these, ideal conditions might be considered unattainable. Nevertheless, Pierce believes that they may be nearly achieved in the rural areas of some Californian valleys in the summer. Accordingly he recommends the addition of electric field monitoring equipment to experiments such as those being carried out by King.

The difficulties with environmental conditions suggest that electric field anomalies are unlikely to be reliable earthquake predictors by themselves, although they could provide valuable support to other observed precursors. It is perhaps worth pointing out, however, that atmospheric electric field monitoring has actually been used in at least one earthquake study. Kondo (*Mem Kakioka Magn. Observ.*, **13**, 11; 1968) recorded occasional field decreases of as much as 20% during the Matsushiro (Japan) earthquake swarm of late 1966. He even suggested that the changes might be related to an enhanced radon content of the atmosphere, but never pursued the idea in detail. □

Ionising helium

from Peter Knight

THE ionisation of an unperturbed helium atom, starting from its ground state, would require an energy equivalent to that of 22 photons from a 1.06 μ m laser. The corresponding "22-photon ionisation" rate would depend upon the 22nd power of the intensity at modest laser intensities, provided that there are no laser temporal coherence effects (due to multimode operation for example) and provided one is far enough away from any possible atomic resonances which would modify the non-linear intensity dependence of the absorption. The fact that 22 photons from a 1.06 μ m laser seem to be needed to ionise a helium

atom when the laser intensity exceeds 10¹⁵ W cm⁻² is, however, a surprise. A group from Saclay has recently reported results on the multiphoton ionisation of rare gases by 30 ps laser pulses at 1.06 μ m (Lompre *et al.*, *Phys. Rev. Lett.*, **36**, 949–952; 1976) which seem to show just that.

Their laser system produces linearly polarised radiation with a linewidth of 0.8 Å centred upon 10 643.5 Å—chosen to avoid atomic resonances. It is operated to generate single pulses of duration 30 ps so that very high peak focused intensities (typically in excess of 10¹⁵ W cm⁻²) may be generated, and so that coherence effects may be avoided. The variation of the number of photoions produced as a function of laser intensity at the focal region is investigated. Then for a single pulse away from an atomic resonance one would expect from the perturbation theory of K-photon multiphoton ionisation a transition rate proportional to the Kth power of the intensity. The following table, taken from Lompre *et al.*'s paper, compares K_0 , the next integer greater than the atomic ionisation energy divided by the laser photon energy, with K_{exp} , the slope of the log-plot of the number of photoions produced as a function of intensity for five rare gases.

Atom	K_0	K_{exp}
Xe	11	11 ± 0.5
Kr	13	13 ± 0.5
Ar	14	14 ± 0.5
Ne	19	20 ± 2
He	22	23 ± 2

K_0 is essentially the minimum number of photons at the laser frequency needed to ionise an unperturbed atom.

So even at the high intensities used by the Saclay group (7×10^{14} W cm⁻² to 1.1×10^{15} W cm⁻²), K_0 and K_{exp} are in good agreement. This is surprising since for these field intensities, the atom certainly is not unperturbed: the peak laser electric field E approaches or even exceeds the internal atomic electric field E_0 responsible for the atomic binding. In such circumstances it is not obvious why the ionisation energy of the unperturbed atom is such a crucial factor. Theoretical work by Keldysh (English translation: *Sov. Phys. JETP*, **20**, 1307; 1965) had suggested that electron tunnelling ionisation rather than multiphoton ionisation would be dominant for $E > E_0$. The Saclay group, approaching $E > E_0$ for the first time in a multiphoton ionisation experiment see no evidence of this yet. If tunnelling occurs present theories predict an intensity-dependent K_{exp} , but this has not been observed. Clearly further experimental work is needed, but already the Saclay group's results indicate a need for new theoretic-

cal approaches to this difficult problem. In particular, the theory of tunnelling urgently needs to be re-examined. □

Junctions and synapses in development

by Eleanor Lawrence and
Miranda Robertson

A meeting on Intercellular Junctions and Synapses in Development was held in Cambridge on April 6-8, 1976, under the auspices of the International Society of Developmental Biologists and University College London. The meeting was organised by Joan Feldman of University College and the proceedings will be published by Chapman and Hall.

SYNAPTOGENESIS and the appearance of other junctions during development raise such different questions that they tend to be considered separately. But answering those questions may involve much the same technology, and it is at the level of technique that the cross-fertilisation encouraged by combining them in the same symposium may prove fruitful.

Gap junctions, the main topic of the meeting, are structures still in search of a function. The structural and permeability properties of the gap junction strongly implicate it as the communication channel for the passage of ions and small molecules between cells, but—except in the myocardium—there is no direct evidence as yet for any physiological function *in vivo*.

By freeze-fracture electron microscopy gap junctions appear as patches of intramembrane particles arranged in hexagonal arrays. The model that was proposed originally for the gap-junction structure by N. B. Gilula (Rockefeller University) was of a dumb-bell shaped protein complex spanning the two apposed membranes and penetrated by a central hydrophilic pore. This model is supported by analysis of X-ray diffraction patterns of isolated gap junctions which has also revealed a six-fold symmetry around the central pore. (D. Goodenough, Harvard Medical School). C. Perrachia (University of Rochester, New York) also proposes a structure of six dumb-bell shaped subunits as an explanation of the different shapes the isolated gap-junctional particles take in the electron microscope.

Electrophoretic separation of isolated gap junction proteins suggests that there are two main subunits but their true molecular weights are far from clear mainly due to proteolysis during

the isolation procedure. Attempts by Goodenough and Don Caspar (Brandeis University) to provide a definitive structure for the gap-junctional particle by the low dose electron microscopy technique used so successfully on bacteriorhodopsin, have unfortunately run into difficulties, since the hexagonal arrays of particles turn out to be not sufficiently regular to give any clear results so far.

A possible structural change which can be correlated with functional uncoupling is the apparent contraction of the gap junctional lattice on fixation, when the particles become more closely packed and smaller in diameter (found independently by Goodenough and Perrachia). Goodenough suggested that that might close the channel by blocking the central pore.

Ionic (electrical) and metabolic coupling in cell culture have been clearly correlated with the presence of gap junctions. Correction of metabolic defects in mutant cells in contact with normal cells, and other biochemical methods, have been used to demonstrate that a wide variety of small cellular molecules such as amino acids, sugars and nucleotides can cross the gap junction: cellular macromolecules do not cross. (John Pitts, University of Glasgow). Birgit Rose (University of Miami) has found a 1,000 molecular weight upper limit for synthetic polypeptides. So far she has found no similar upper limit for synthetic oligosaccharides which are apparently still going through at molecular weights of 1,300. In *Chironomus* at least, the permeability of the gap junction can be regulated by Ca^{2+} , which at sufficiently high concentrations at the junction blocks the passage of an electrical current (Rose).

Although the idea that gap junctions have an important role as a communication channel between like cells during development is attractive it is thus far unsupported by any convincing evidence. J.-P. Revel (Caltech), who claimed to have found his own results almost impossible to interpret, remarked pessimistically that the existence of a fine telephone system doesn't necessarily mean that there's anybody talking down it, or even if there is, that they're talking sense. He finds that during neurulation in the chick embryo, not only do the gap junctions present in the epiblast before invagination of the neural groove disappear as the neural groove develops but the tight junctions in that region also become disrupted and all that can be seen when the neural tube closes are scattered groups of a few particles where the tight junctions should be. It is impossible to tell whether those are tight junctions disappearing or gap junctions forming or whether the gap

and tight junctions are transmutable.

Anne Warner (Royal Free Hospital, London) has elected to follow the changing patterns of electrical coupling in developing axolotl and *Xenopus* embryos. She presented a beautifully clear demonstration of electrical coupling changes during somite formation in the axolotl and *Xenopus*. In both embryos the unsegmented mesoderm cells are strongly coupled to each other. Prospective somite cells become uncoupled before or during somite formation and in *Xenopus* the cells in the newly-formed somite recouple not only to each other but to adjacent somites as well. In the axolotl the formed somites do not couple to each other. Gap junctions between coupled cells have been demonstrated in this system (Blackshaw and Warner, *J. Physiol., Lond.*, **255**, 209-230; 1976).

S. W. de Laat (Hubrecht Laboratory) directly challenged the assumption that ionic coupling seen between the cells of the first cleavage to fifth cleavage *Xenopus* embryo is mediated by gap junctions, by suggesting that the electrical coupling is due to the generally lower permeability of the intercellular membranes relative to the outer bounding membranes rather than to the presence of specialised low resistance junctions (gap junctions) in the cleavage membrane.

But during discussion it was pointed out that although these intercellular membranes are relatively particle-free, even the few particles that can be detected could account for the ionic coupling if they were acting as individual communication channels.

R. S. Decker (University of Dallas) has developed a simple and elegant system for studying the formation of gap junctions during differentiation. He has followed the progress of junction formation during hormone-induced differentiation *in vivo* (hypophysectomised *Xenopus* embryos responding to thyroxine) and *in vitro* (adrenal cortical tumour cells responding to ACTH) and has demonstrated that both RNA and protein synthesis are required.

The existence of defined junctional mutants could aid the analysis of the functional significance of the gap junction considerably. Pitts reported that no single-step mutations have yet been found. W. Loewenstein (University of Miami) has been studying the behaviour of somatic cell hybrids between communication competent normal human cells (which possess gap junctions) and communication incompetent mouse cells (lacking junctions) and finds that the early heterokaryons resemble the normal parent. But as the human chromosomes are gradually lost over the generations the cells revert to communication incompetence. Loewen-

stein has been unable to ascribe reversion to the loss of any particular human chromosome or chromosomes but believes that not more than two are involved.

The principal problem in synaptogenesis is not its *raison d'être* but its mechanism—how do innervating fibres recognise their target cells, how specific is that recognition and what part is played by competitive interactions between innervating fibres? C. Sotelo (INSERM) reviewed morphological evidence from mouse cerebellar mutants that in the absence of the normal postsynaptic sites, growing nerve fibres will make alternative synapses and conversely that in the absence of their normal innervation postsynaptic structures can attract nerve fibres which would not normally reach them.

The existence of relatively non-specific specialised sites intrinsic to the membrane of postsynaptic cells in the CNS was emphasised by G. Raisman (MRC Mill Hill) who described the reinnervation of denervated adult rat superior cervical ganglion cells by the vagus and the hypoglossal nerves at the sites of the postsynaptic thickenings originally occupied by preganglionic nerve fibres.

The molecular nature of the recognition system for postsynaptic membrane is unknown, although one obvious possibility is the neurotransmitter receptors themselves. Most attempts to test that possibility in the central nervous system have failed. But J. Freeman (Vanderbilt University) has succeeded in showing that the retinotectal system in *Bufo* is cholinergic, and claims that by applying the cholinergic receptor blocker α -bungarotoxin to small areas of the developing optic tectum he can create localised defects in the electrophysiological map of the visuo-tectal projection.

Detailed studies on the behaviour of receptors are much easier at the neuromuscular junction, where it is believed that the increased density of receptors at the postsynaptic membrane is a consequence of innervation rather than its cause. G. Fischbach (Harvard University) however presented evidence that chick skeletal muscle cells *in vitro* develop areas of high receptor concentration in the absence of innervation. These receptor "hot spots" are detected by intracellular recordings from the muscle as areas of heightened sensitivity to focal stimulation with ACh, which confirms Nirenberg's report that local concentrations of α -bungarotoxin receptor binding sites exist on muscle cells *in vitro*.

The stability of concentrations of receptor sites *in vivo* was emphasised by C. Slater (Newcastle General Hospital), who finds that the peaks of sensitivity that develop in denervated

adult rat soleus on reinnervation with the superficial fibular nerve persist for at least a week after section of the fibular nerve.

The question of competitive interactions in the specification of neuromuscular junctions formed in developing mammals was addressed by D. van Essen (University College London), who reported that the multiple innervation found in neonatal soleus is eliminated by 2–3 weeks after birth, through the withdrawal of supernumerary terminals by the motor axons. This shrinkage occurs however even when half the competing terminals are removed by the early section of one of the ventral roots, and some muscle fibres are left vacant. In experiments in which an immature muscle was denervated and reinnervated by a foreign nerve which was then forced to compete for synaptic sites with the regenerating terminals of the original nerve, multiple innervation persisted only where the synapses were at least 1 mm apart.

The resolution of problems in synaptogenesis seems likely to be found in the properties of the membranes; the properties of gap junctions will remain a series of tantalising hints until someone can define a disruptive effect of blocking them *in vivo*. □

Radiological data collection

from a Correspondent

A meeting on Radiological Protection Data: Collection, Recording and Use, organised by the Society for Radiological Protection was held in London on April 6, 1976.

A NATIONAL register of radiation workers is to be set up in this country, S. Rae, Assistant Director (Medical) of the National Radiological Protection Board, told the meeting. Negotiations for establishing the register, initially to cover workers in the nuclear energy industry, are now being conducted. It is hoped to extend it to include for example, industrial radiographers and miners exposed to radiation.

The purpose of setting up such a national register will be to carry out an epidemiological study of cancer mortality in radiation workers and to look for effects of occupational radiation exposure on the natural rate of cancer induction. The register will follow, for at least 25 years, the radiation exposure of a large population of radiation workers and will match this with information obtained from the records of the Office of Population Censuses and

Surveys about the age at death and the cause of death of these workers.

He indicated that calculations, based on current estimates of rates of cancer induction per million individuals per rad, suggest that even with a postulated study of 10,000 workers followed for 25 years it is most unlikely that there would be a detectable increase in the number of cancer deaths from occupational exposure to radiation at the currently existing level. If, however, no excess mortality is demonstrated by such a study then we can conclude that the risk coefficients we assume and the levels of radiation exposure we endeavour to keep to are justified, and this information is well worth knowing.

R. H. Clarke (Berkeley Nuclear Laboratories) described some work he had been carrying out in conjunction with Professor Mayneord on computer modelling of non-linear dose-response relationships. He quoted considerable published evidence for a non-linear, peaked, response between dose and risk and then discussed the implications of such a relationship in radiological protection. From his studies, Clarke was unable to show that the absolute expectations of malignant conditions were significantly greater for these non-linear dose-response functions than for the conventional linear hypothesis on which the recommendations of the International Commission on Radiological Protection are based.

The familiar film-badge which radiation workers have been used to wearing for so many years in this country will for many of them soon become a thing of the past. The National Radiological Protection Board is shortly to introduce a new thermoluminescent dosimeter (TLD) for the majority of its customers and a prototype of the dosimeter was on display for the first time at the meeting. The dosimeter consists of a metal plate carrying two inserts of lithium fluoride-impregnated PTFE discs. The dosimeter is wrapped in black polythene film and worn in a black plastic holder.

The advantage of a TLD is that it readily lends itself to automation in dosimeter issue and readout, and in dose record keeping. E. Greenslade (NRPB) told the meeting about the relatively simple interim dose record keeping service, based on film-badges, at present operated by the NRPB. This service covers some 3,800 workers at 330 establishments. The new TLD will form the basis of an automated dosimeter issue and record keeping service which will be capable of holding up to 200,000 records. L. Salmon (Atomic Energy Research Establishment, Harwell) described the data base system, DIRK, which had been developed at Harwell for the NRPB's automated TLD service. □

articles

Evolution of type C viral genes: evidence for an Asian origin of man

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Old World monkeys and apes, including man, possess, as a normal component of their cellular DNA, gene sequences (virogenes) related to the RNA of a virus isolated from baboons. A comparison of the viral gene sequences and the other cellular sequences distinguishes those Old World monkeys and apes that have evolved in Africa from those that have evolved in Asia. Among the apes, only gorilla and chimpanzee seem by these criteria to be African, whereas gibbon, orang-utan and man are identified as Asian, leading us to conclude that most of man's evolution has occurred outside Africa.

THE endogenous, genetically transmitted type C viruses isolated from baboons can be used to generate nucleic acid probes which provide a new approach to the study of primate evolutionary relationships¹. With DNA transcripts of the baboon RNA virus genome, related nucleic acid sequences can be detected as normal constituents of the cellular DNA of baboons, other Old World monkeys, and apes, including man. The amount of base-pair mismatching, as determined by the thermal stability of DNA-DNA hybrids or by the final extent of hybridisation, correlates well with the classification of primates based on anatomical considerations and the fossil record^{2,3}. We have explored this in more detail by comparing the virogene sequences and the overall non-repetitive cellular DNA for 11 Old World monkey and 6 ape genera, including man.

African Old World monkeys (8 genera, including several baboon species) and African apes (gorilla and chimpanzee), possess virogenes that are useful in the taxonomic classification of these animals. The Asiatic Old World monkeys (macaques, *Cynopithecus*, and langurs), and the Asiatic apes (gibbon, siamang, and orang-utan) contain virogene sequences that possess much less sequence homology (relative to the baboon) than those of the African primates. When human DNA is examined, its extent of virogene sequence divergence agrees with that obtained for the Asian apes and is substantially different from that of either African ape.

Nucleic acid homologies among primate DNAs

The extent of reassociation and the thermal stability of DNA strands derived from different species are a measure of their evolutionary relationships⁴⁻⁶. Non-repeated sequences comprise a large fraction of mammalian DNA; each sequence is believed to occur only once per haploid genome⁷. This DNA is therefore suitable for determining the extent of nucleotide diversity that has accumulated since two species diverged from their last common ancestor.

Eleven genera of Old World monkeys (Cercopithecidae)

belonging to two subfamilies (Cercopithecinae and Colobinae) and six genera of apes (including man) were examined for the extent of homology of their cellular DNA with that of a baboon, *Papio cynocephalus*. The baboon is the index species for organising the Old World primates to facilitate comparison with the data obtained for the virogene sequences. Table 1 shows that the cellular DNAs of other baboon species are, as expected, most closely related to *P. cynocephalus* DNA; *P. anubis* shows the greatest sequence homology to *P. cynocephalus* while *P. papio* and *P. hamadryas* DNA possess somewhat less. The Cercopithecinae most closely related to the baboon group include the gelada, mandrill, macaques and mangabeys. Guenon, vervet and patas monkeys are more distantly related. The cellular DNAs of the more primitive subfamily Colobinae (the leaf-eating monkeys) show less nucleic acid sequence homology to baboon DNA than does the cellular DNA of any of the monkeys of the subfamily Cercopithecinae. The apes and man form a distinct group since their DNA is even more distantly related to baboon DNA than is that of any Old World monkey; the cellular DNAs of the various ape genera hybridise to approximately 50% of baboon cellular DNA with the conditions used. Three genera of New World monkeys were also examined; these are the most distantly related of the primates tested (approximately 35% homology).

The thermal stability of nucleic acid hybrids was also used as an index of the degree of base-pair mismatching between the primate DNA strands (Table 1). Mismatching results in the formation of hybrids that melt at a lower temperature; the effect of mismatched base-pairs on the thermal stability has been estimated at between 0.7 and 1.7 °C per 1% altered pairs⁸. The decrease in thermal stability between the homologous baboon cellular DNA hybrid and the hybrids formed between baboon DNA and the DNA of the other primate species is expressed as ΔT_m . It is evident that the cellular DNAs of those primates that show progressively less homology to baboon DNA also show a decrease in their thermal stability relative to the homologous baboon DNA hybrid. Therefore, the decrease in the final extent of nucleic acid homology is most likely due to an accumulation of base-pair mutations, reflecting an increasing phylogenetic distance from the baboon.

Table 2 shows the thermal stability of ape and human non-repetitive cellular DNA hybrids. Radioactively-labelled cellular DNA from diploid cultures of human, chimpanzee, and gorilla cells was hybridised to cellular DNA from cell cultures and tissues of several representatives of each species. Chimpanzee, gorilla and human cellular DNA all yield a $\Delta T_m R$ of approximately 2.3–2.6 °C when hybridised to each other. More distantly related to these three apes are the orang-utan (4.5 °C $\Delta T_m R$), the gibbon and siamang (6.3–6.5 °C $\Delta T_m R$), and the baboon (8.6–9.3 °C $\Delta T_m R$). The $\Delta T_m R$ values shown in Table 2 for ape-baboon DNA hybrids are the same as the values shown for the baboon-ape hybrids in Table 1. There is therefore a

Table 1 Nucleic acid homology and thermal stability between baboon cellular DNA and the DNA from various primate species

Species	% hybrid	Baboon cellular DNA ΔT_m (°C)	ΔT_{mR} (°C)
Old World monkeys			
Cercopithecidae			
Cercopithecinae			
Baboon (<i>Papio cynocephalus</i>)	100	—	—
(<i>P. anubis</i>)	99	0.1	0.2
(<i>P. papio</i>)	96	0.4	0.7
(<i>P. hamadryas</i>)	93	0.7	1.1
Gelada (<i>Theropithecus gelada</i>)	90	1.0	1.8
Mandrill (<i>Mandrillus sphinx</i>)	88	1.2	2.1
Celebes ape (<i>Cynopithecus niger</i>)	84	1.2	2.3
Rhesus (<i>Macaca mulatta</i>)	86	1.4	2.7
Stump-tailed macaque (<i>M. arctoides</i>)	85	1.3	2.5
Pig-tailed macaque (<i>M. nemestrina</i>)	82	1.4	2.9
Crab-eating macaque (<i>M. fascicularis</i>)	85	1.4	2.8
Mangabey (<i>Cercocebus atys</i>)	84	1.4	2.6
(<i>C. torquatus</i>)	82	1.4	2.6
Guenon (<i>Cercopithecus campbelli</i>)	77	2.1	3.9
Vervet (<i>C. pygerythrus</i>)	75	2.2	4.4
Patras (<i>Erythrocebus patas</i>)	71	2.4	4.8
Colobinae			
Colobus (<i>Colobus guereza</i>)	64	3.2	6.5
Langur (<i>Presbytis obscurus</i>)	62	3.3	6.4
Apes			
Gibbon (<i>Hylobates lar</i>)	50	4.5	9.0
(<i>H. agilis</i>)	49	4.4	9.0
Siamang (<i>Symphalangus syndactylus</i>)	50	4.4	8.9
Orang-utan (<i>Pongo pygmaeus</i>)	51	4.4	8.9
Chimpanzee (<i>Pan troglodytes</i>)	54	4.8	9.3
Gorilla (<i>Gorilla gorilla</i>)	50	4.9	9.5
Man (<i>Homo sapiens</i>)	56	4.7	9.2
New World monkeys			
Woolly (<i>Lagothrix spp.</i>)	31	8.2	15.2
Capuchin (<i>Cebus spp.</i>)	37	8.5	15.0
Howler (<i>Alouatta spp.</i>)	33	8.0	15.0

The DNA of a baboon lung cell strain was labelled with ^3H -thymidine (120 μCi per ml of cell culture media) for 72 h and extracted²⁰. "Non-repetitive" cellular DNA was isolated by removing the highly reiterated DNA sequences that anneal by a C_0t of 200 (40% of the total DNA) by fractionation on hydroxyapatite¹. Cellular DNA was extracted from tissues and cell cultures, and sonically treated to a mean size of 6–8S as determined by centrifugation on alkaline sucrose gradients²⁰. DNA–DNA hybridisations were incubated at 65 °C in reaction mixtures containing 0.01 M Tris, pH 7.4, 0.75 M NaCl, 2×10^{-3} M EDTA, 0.05% SDS, 40,000 c.p.m. of ^3H -thymidine-labelled baboon cellular DNA (0.2 μg) per ml and 2–4 mg of cellular DNA per ml. Hybridisations were started by heating the mixtures to 98 °C for 10 min, cooling on ice to 4 °C, and incubating at 65 °C. At various times, 0.025-ml portions were removed and frozen at –80 °C. The percentage hybrid is the saturating normalised value obtained after digestion of the hybrids with the single-strand specific nuclease S_1 (refs 36, 37). All hybridisations were carried out to a C_0t value^{7,38} of 4×10^4 . The actual final extent of *P. cynocephalus* cellular DNA hybridisation to its non-repetitive cellular ^3H -DNA was 76%. The temperature at which 50% of the hybrids are dissociated (T_m) was 86 °C for the homologous *P. cynocephalus*–*P. cynocephalus* hybrid. The ΔT_m is the difference in T_m between the other DNA–DNA hybrids and the T_m of the homologous hybrid. ΔT_{mR} is the thermal stability difference for all the non repeated DNA⁴, and is the ΔT_m value corrected for the final percentage of hybridisation of the cellular DNA to the labelled baboon DNA. This correction is based on the assumption that the difference between the various primate DNAs is due to divergence from common ancestral nucleic acid sequences, and not to the addition or deletion of large amounts of DNA unrelated to that in the species being tested.

close agreement in ΔT_{mR} values for the various reciprocal hybrids tested. The siamang and gibbon cellular DNAs possess as much nucleic acid sequence homology (2.5 °C ΔT_{mR}) as chimpanzee, gorilla and human DNA share with each other.

The thermal stabilities of the various non-repetitive cellular DNA hybrids were used to construct a phylogenetic tree of primate evolution (Fig. 1). Since there is an apparent effect of generation time on the rate of accumulation of base-pair mutations in DNA^{4,9,10}, divergence times for certain of the apes have been adjusted for the time needed to reach a fertile state, as explained in the legend. In addition, all the divergence times shown depend on the choice of 30 Myr as the time when Old World monkeys and apes initially diverged¹¹. The data show the gelada closely related to the baboon and are consistent with a model in which the baboon, mangabey and macaque lineages have been derived from a common ancestor somewhat more recently (7–8 Myr ago) than the lineage leading to the *Cercopithecus* and *Erythrocebus* group (12 Myr). The subfamily Colobinae (langur and colobus) seems to have diverged from the other Old World monkey subfamily Cercopithecinae approximately 18 Myr ago. Hybrid animals have been obtained by breeding different species of *Papio* (*P. anubis* and *P. hamadryas*, for example) and different species of *Macaca* (*M. mulatta* and *M. nemestrina*), as well as *Papio* and *Theropithecus*, and *Papio* and *Macaca*¹². The *Papio*–*Theropithecus* hybrids are

apparently fertile, while the *Papio*–*Macaca* hybrids are reported to be sterile¹². These data support the conclusion that the *Papio*–*Theropithecus* divergence is more recent than that of *Papio* and *Macaca*, and suggest that a ΔT_{mR} difference of 2 °C for the reassociation of DNA hybrids may represent the upper limit in anthropoid primates for genetic compatibility leading to the production of fertile offspring.

As is evident from Fig. 1, man is quite closely related to the chimpanzee and gorilla. The human–chimpanzee and human–gorilla "non-repeated" cellular DNA hybrids dissociate with a ΔT_{mR} of approximately 2.4 °C (ΔT_m 1.3 °C) lower than the dissociation temperature obtained for the reannealing of human DNA. The divergence between chimpanzee, gorilla and man emerges therefore as a trichotomy with a common ancestor 12 Myr ago (8 Myr if the data are not corrected for the longer generation time of this group). Our data do not reveal any two of these three species as being closer to each other than the third (see Table 2). Both African apes, then, are equally close to man; our next closest relatives, but clearly more distant than the African apes, are the orang-utan (diverging 18 Myr ago) and the gibbons (diverging 26 Myr ago). The gibbon and siamang are closely related to each other, having last had a common ancestor about 6–8 Myr ago.

The data obtained correlate closely with those of other investigators who used DNA reassociation techniques^{4–6,9} or

amino acid and immunological data¹³⁻¹⁷ to classify the primates, as well as with some of the phylogenetic relationships established on the basis of anatomical considerations and the fossil record^{2,3,11}. The nucleic acid sequence homology of ape cellular DNA supports the classification proposed on an immunological basis by Goodman and Moore¹⁷ that places man, chimpanzee and gorilla in one subfamily (Homininae) since they clearly had a common ancestor more recently than any of these three species had with the orang-utan or with the gibbons. The genetic distance between man, chimpanzee and gorilla as measured by the decrease in thermal stability of nucleic acid hybrids ($\Delta T_m R$) is comparable, for example, with that found between gibbon and siamang, or between baboon, rhesus and mangabey (see Table 1).

The actual evolutionary dates for the divergence of the various primate species depend on the choice of 30 Myr for the ape-Old World monkey divergence. If this date is shown to be radically wrong (most likely as an underestimate) by future palaeontological discoveries, the relative relationships among the various primate species would nevertheless remain the same. For example, the extent of nucleic acid sequence divergence among chimpanzee, gorilla and man is three to four times less than that between any of these three Homininae and the Old World monkeys.

Nucleic acid homologies among primate type C viral genes

All Old World monkeys and apes have previously been shown to contain endogenous primate type C virogenes as an integral component of their cellular DNA¹. We therefore examined these primates for the extent of homology of their type C virogenes to the type C virus isolated from baboons. The baboon type C virus used in these studies was first isolated and subsequently propagated in non-primate cell lines (dog, bat, or mink)^{18,19} to eliminate the possibility that the radioactively-labelled DNA probes prepared from the baboon viral RNA might contain other primate cellular sequences.

Figure 2 shows that there is a decrease in the final extent of hybridisation and in the thermal stability of hybrids formed

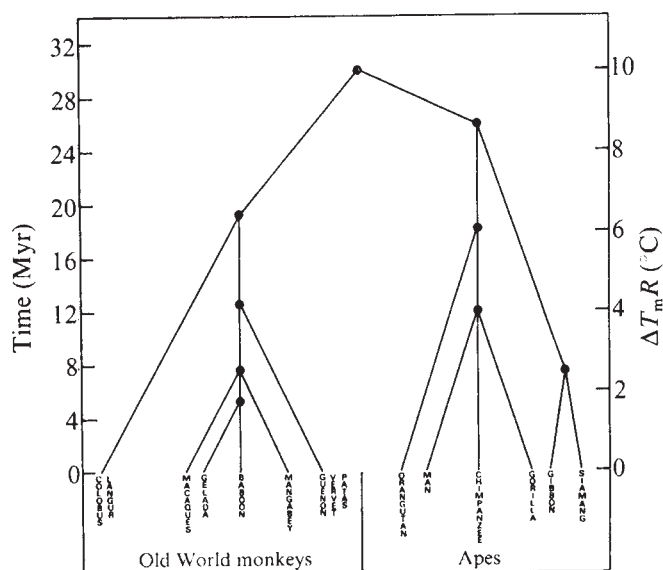


Fig. 1 Phylogenetic tree depicting relationships among the cellular DNAs of Old World monkeys and apes. The $\Delta T_m R$ ($^{\circ}\text{C}$) of the various DNA-DNA hybrids shown in Tables 1 and 2 are plotted as a function of years of evolution. The points shown depend on the choice of 30 Myr for the time when Old World monkeys and apes diverged¹¹. For the Old World monkeys (generation time 4-6 years³⁹), a value of $1^{\circ}\text{C } \Delta T_m R$ equal to 3 Myr of evolution was chosen based on the fact that the $\Delta T_m R$ between Old World monkey and ape DNA is approximately 9°C . This relationship was also employed for the gibbon and siamang (generation time approximately 6 yr). For the longer lived higher apes, the values were adjusted as follows: orang-utan ($1^{\circ}\text{C } \Delta T_m R$ equal to 4 Myr) and chimpanzee, gorilla, and man ($1^{\circ}\text{C } \Delta T_m R$ equal to 5 Myr) (generation time of approximately 8-10 yr). The generation time is taken as the time needed to reach a fertile state; the present-day values are assumed to provide an estimate of the generation time of the ancestors of the species⁴⁰. A more comprehensive discussion of the correction for differences in the generation times among present-day primates is in ref. 10.

Table 2 Thermal stability of hybrids formed between ape non-repetitive cellular DNAs

Species	Cellular DNAs $\Delta T_m R$ ($^{\circ}\text{C}$)			Gibbon
	Chimpanzee	Gorilla	Man	
Chimpanzee	—	2.4	2.3	6.3
Gorilla	2.6	—	2.4	6.5
Man	2.4	2.6	—	6.9
Orang-utan	NT	4.5	NT	NT
Gibbon	6.5	6.5	6.4	—
Siamang	6.3	NT	6.4	2.5
Baboon	9.3	8.6	9.0	9.1

Chimpanzee, gorilla, human and gibbon cell cultures were each labelled with ^3H -thymidine and the non-repetitive cellular DNA isolated and hybridised to the cellular DNA of the species listed as described in the legend to Table 1. The chimpanzee cell cultures (obtained from Dr C. J. Gibbs) were derived from brain cells. One line of chimpanzee liver cells obtained from a commercial source and used in our initial experiments characterising the baboon probe²⁰ has been identified as human in origin. The $\Delta T_m R$ values for the various DNA-DNA hybrids are listed. The ΔT_m values are approximately one-half the values shown; for example, the ΔT_m value for the human-chimpanzee hybrid was 1.4°C . The temperature at which 50% of the hybrids are dissociated (T_m) ranged between 84°C to 88°C for the homologous DNA-DNA hybrids in various experiments. The T_m values were derived from six separate experiments in which hybrids were melted at 1°C intervals over a range of $\pm 7^{\circ}\text{C}$ from the T_m .

between the baboon viral DNA probe and primate cellular DNA that is a function of phylogenetic distance from the baboon. The hybrid with the highest thermal stability is obtained with the cellular DNA of *P. cynocephalus*, the species from which the baboon type C virus was isolated. The DNA from three other species of baboons form hybrids with the baboon type C DNA probe that are slightly less stable (ΔT_m values range from 0.3 to 2.2°C lower). The cellular DNAs from other species of Old World monkeys form hybrids that contain even more extensive base-pair mismatching. The reduced thermal stabilities obtained with the Old World monkey DNAs also indicate that the partial homologies detected between the baboon type C viral probe and the DNAs from these primates are a result of alteration by base-pair substitution or by rearrangements, of much, and perhaps all, of the viral genome, and not to the strict preservation of a large segment of the viral genome.

A striking finding, however, is that the decrease in nucleic acid homology and thermal stability for the type C viro gene sequences does not always parallel the decrease in thermal stability of the non-repetitive cellular DNA. For example, even though the macaques and mangabeys are approximately equally distant phylogenetically from the baboon ($2.6^{\circ}\text{C } \Delta T_m R$; see Table 1 and Fig. 1), the viro gene sequences of these animals are quite different, forming hybrids with baboon viral DNA that melt 8.2°C and 5.0°C lower, respectively. Even more striking are the data obtained with the Colobinae. *Colobus* and *langur* cellular DNA possess an equivalent

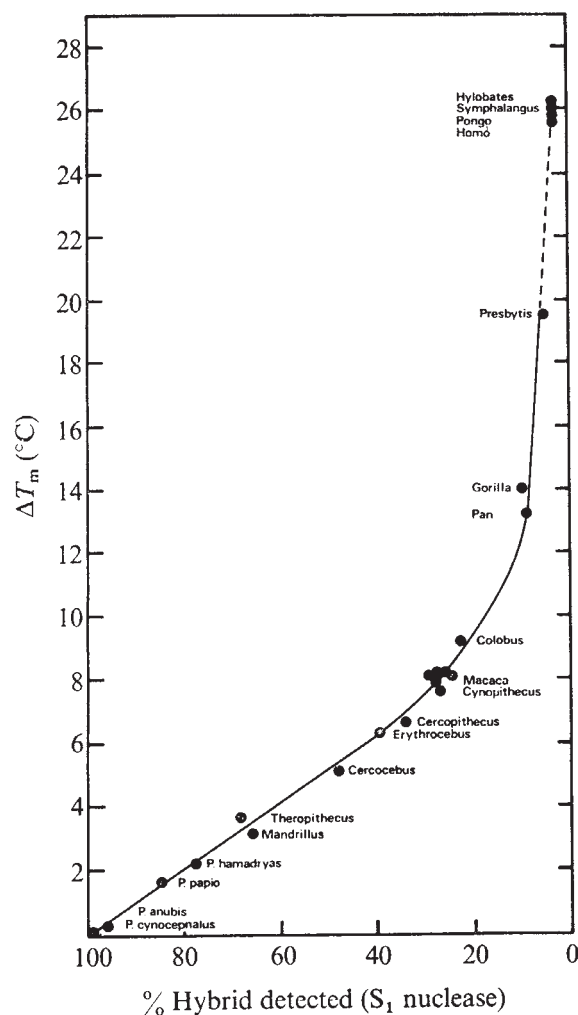


Fig. 2 Hybridisation of baboon type C viral DNA probe to primate cellular DNAs. ³H-thymidine-labelled viral DNA probes were prepared as follows: the baboon viruses^{18,19} were propagated in either a canine thymus (FCf2Th) or a bat lung (TbLu) cell line from which supernatant media was collected every 3 h for 1 d and stored at 2 °C. The supernatant was clarified of cellular debris and the virus banded on sucrose gradients, pelleted, and used immediately in an endogenous reverse transcriptase reaction. Reaction mixtures were incubated at 37 °C for 2 h and contained 0.04 M Tris, pH 7.8, 0.06 M KCl, 6 × 10⁻³ M magnesium acetate; 2 × 10⁻³ M dithiothreitol; 0.02% (v/v) Triton X-100; 30 μg ml⁻¹ actinomycin D; 5 × 10⁻⁵ M ³H-TTP (50 Ci mmol⁻¹), and 2 × 10⁻³ M each of dATP, dCTP, and dGTP. These conditions have been shown to result in the synthesis of long, representative DNA copies of the viral genome⁴¹⁻⁴³. The ³H-DNA baboon probe was deproteinised and purified as described previously²⁰. The specific activity of the ³H-DNA was 1.8 × 10⁷ c.p.m. μg⁻¹. The ³H-DNA probes contained 65–86% of their respective 70S viral RNA sequences at a ³H-DNA: ³²P-viral RNA molar ratio of 1.0 (ref. 20), indicating that they contain most of the sequences present in viral RNA and that these sequences are in proportions similar to their content in 70S RNA. Several of these baboon probes were subsequently further purified by hybridising them to 70S or 35S viral RNA extracted from baboon virus grown in the cells of a non-primate species different from the one in which the virus had been grown. Probes prepared from the baboon virus grown in the bat lung cell line were cycled against RNA extracted from the virus grown in the canine thymus cell line. In some experiments the reciprocal cycling was performed: virus grown in the canine thymus cell line was cycled against RNA extracted from the virus grown in the bat cell line. The non-hybridised portion of the baboon ³H-DNA probe was removed by digesting with S₁ nuclease. These probes were then treated with alkali to hydrolyse the RNA, and deproteinised and purified as described²⁰. Viral probes treated in this manner hybridised to 90–100% of baboon viral RNA. DNA-DNA hybridisations were incubated as described in the legend to Table 1, and the extent of hybridisation determined with the single-strand specific nuclease S₁. The normalised final percentage values obtained after hybridising the various primate cellular DNAs to the baboon viral probe are shown. The ΔT_m values obtained for the DNA-DNA hybrids relative to the homologous baboon viral ³H-DNA-*Papio cynocephalus* cellular DNA hybrid are listed on the ordinate. The final percentage hybridisation values of the baboon viral probe to gibbon, siamang, orang-utan and human DNA are too low (3–4%) (see Table 3) for the ΔT_m to be determined experimentally; these values have been extrapolated from the curve as shown.

amount of nucleic acid sequence dissimilarity relative to baboon cellular DNA (6.4–6.5 °C ΔT_m, Table 1), but the virogenes of these two species hybridise to 23% and 6% of the baboon viral probe, respectively (Fig. 1, Table 4). Finally, although the DNA of all six genera of apes are indistinguishable relative to baboon cellular DNA (8.9–9.5 °C ΔT_m), two of the apes, chimpanzee and gorilla, contain virogene sequences that are much more closely related to baboon virogene DNA than are those of the other apes (gibbon, siamang, orang-utan) or man. In fact, chimpanzee and gorilla cellular DNA consistently hybridise more to the baboon virogene probe than does the DNA of the langur, an Old World monkey. The hybridisation values of gibbon, siamang, orang-utan and human DNA to baboon viral DNA as detected by S₁ nuclease are too low (3–5%) for the ΔT_m values to be determined experimentally; they are extrapolated from the curve as shown in Fig. 2.

Earlier studies on primate evolution using the baboon viral probe^{1,20,21} focused on the observation that the baboon virus was, in fact, an endogenous primate virus and that related nucleic acid sequences could be detected in various other Old World monkey tissues. The final extents of hybridisation to ape DNAs were much lower than those obtained to Old World monkey DNAs but significantly higher than those obtained to New World monkey, prosimian, or other mammalian DNA. The differences in final extent between the various ape DNAs were difficult to determine based on only a few S₁ nuclease determinations¹. In one previous study, the source of chim-

panzee DNA was a tissue culture line (chimpanzee liver cells, obtained from Flow Laboratories, Rockville, Maryland) which provided an apparent discrepancy in the data²⁰. Further analysis revealed the cell line DNA to be human, most probably HeLa, and not chimpanzee.

Since only a low degree of homology is obtained with the baboon viral probe to ape DNA using stringent hybridisation assay conditions (such as S₁ nuclease), a large number of experiments is needed to obtain statistically significant differences. The final extent of hybridisation (from several separate experiments) of the baboon viral probe to colobus and langur DNA and to chimpanzee, gorilla, gibbon, orang-utan and human DNA is shown in Table 3. Although the final hybridisation levels obtained with langur, gibbon, and human DNA are low, they are significant when compared with the hybridisation values obtained with four non-primate mammals (cow, hippopotamus, pig and rabbit) as well as with the non-mammalian salmon sperm DNA. The DNAs from a total of 36 human tissues have been separately examined. Most show a small increase in the final extent of hybridisation when compared with non-primate DNAs, some do not hybridise significantly above the levels found with the non-primate controls, while still others yield what seem to be unexpectedly high final hybridisation values to the baboon viral transcripts. The latter DNAs (3 of 36) have been obtained from tissues of individuals with tumours. The basis for the apparent occasional "higher reactors" among human DNA samples requires further study and will be the subject of a separate report. Nevertheless,

the large differences in the final extent of hybridisation of colobus and langur DNA and of chimpanzee and human DNA to the baboon type C viral probe are clearly evident. With a DNA transcript prepared from an endogenous domestic cat virus (RD-114) that is partially related to the baboon virus, a higher final extent of homology has also been obtained to gorilla and chimpanzee DNA than to human or gibbon cellular DNA²².

Hybrids with more extensive mismatching can be detected by lowering the stringency of the annealing conditions²³; this permits the detection of partially homologous sequences in more distantly related species⁴. Table 3 shows data obtained with the baboon viral probe when the hybridisation conditions are modified by decreasing the temperature to 56 °C, the salt concentration to 0.50 M Na⁺, and by using hydroxyapatite rather than S₁ nuclease, thus allowing partial hybrids to be detected as double-stranded molecules. Although the background values obtained with non-primate DNA increase, the higher final extent of hybridisation to chimpanzee and gorilla DNA compared with gibbon, orang-utan and human DNA are still evident.

A higher final extent of hybridisation to the various Old World monkey DNAs can also be obtained by changing the S₁ nuclease incubation conditions. For example, by decreasing the temperature of the S₁ nuclease incubation from 45 °C to 37 °C and by increasing the monovalent cation concentration from 0.15 M to 0.30 M, the final extent of hybridisation of the baboon viral probe to mangabey DNA increases from 50% to 85%, and the value to rhesus and other macaque DNAs increases from 25% to 60%. The relative relationships among the various primate viro gene sequences remain the same.

Differences in African and Asian primate type C viral genes

The Old World monkeys and apes inhabit both Asia and Africa. All the genera of the Old World monkey subfamily Cercopithecinae inhabit Africa with the exception of various macaque species and the closely related celebes ape (*Cynopithecus*), which inhabit Asia. Table 4 lists representative species from the subfamily Cercopithecinae that have evolved in both areas and whose DNA has diverged to a comparable extent from baboon cellular DNA (as shown by their $\Delta T_m R$ values). The cellular DNA of those animals whose habitat is Africa hybridises to a much greater extent to the baboon viral DNA probe. The DNA extracted from two species of mangabeys, for example, hybridises 48% and 51% to the baboon type C viral DNA probe, whereas the DNA extracted from four different species of macaques hybridises only 25–28%. The use of hydroxyapatite to detect these hybrids raises the final extent of hybridisation to 65–70% for mangabey DNA and to 45–50% for rhesus DNA, but the clear differences between these primates remain. Two genera from the other Old World monkey subfamily, the Colobinae, were also examined. The cellular DNA from the African species, the colobus, hybridises to 23% of the baboon viro gene probe, whereas the Asian langur cellular DNA hybridises to only 5% of the probe. Finally, among the apes, the cellular DNA of chimpanzee and gorilla (which inhabit Africa) hybridise 9% and 10%, respectively, to the baboon viro gene probe. The gibbon, siamang and orang-utan, which inhabit Southeast Asia, hybridise to a much lower extent to the baboon viral probe, as does the human DNA.

Table 3 Detection of endogenous type C viro gene sequences in various primate and human tissues

Species	No. of individuals tested	No. of tissues tested	No. of Expts	% Hybrid S ₁ nuclease*		
				Avg.†	s.d.	% Hybrid minus background‡
Colobus	2	3	18	27.8	±2.6	24.1
Langur	1	2	12	9.1	±1.5	5.4
Gorilla	2	5	15	14.0	±1.4	10.3
Chimpanzee	5	11	44	13.5	±3.1	9.8
Gibbon	8	14	28	6.8	±1.3	3.1
Man	36	36	68	7.0	±2.6	3.3
Cow	3	3	14	3.5	±1.0	0
Hippopotamus	1	2	6	3.8	±0.8	0
Pig	5	10	18	4.3	±0.9	0
Rabbit	2	6	12	3.3	±0.9	0
Salmon	1	1	10	1.5	±0.7	0
% Hybrid hydroxyapatite ‡						
Chimpanzee	2	3	6	26.7	±4.7	18.7
Gorilla	1	2	4	27.8	±2.5	19.8
Gibbon	2	2	6	15.2	±2.0	7.2
Orang-utan	1	1	4	14.7	±4.8	6.7
Man	5	5	12	15.8	±3.1	7.8
Cow	1	1	12	8.0	±2.1	0
Pig	2	2	5	8.5	±2.2	0
Rabbit	1	2	12	7.5	±2.0	0
Salmon	—	—	5	2.7	±0.7	0

*Cellular DNA was extracted from various tissues of the species listed²⁰; salmon sperm DNA was obtained commercially. ³H-DNA probes prepared from the baboon type C virus grown in a bat lung cell line (TbLu) or in a canine cell line (FCf2Th) were hybridised at 65 °C in 0.75 M NaCl to the various cellular DNAs as described in the legend to Table 1. The extent of hybridisation was determined by digestion of the hybrids with S₁ nuclease^{26,27}.

†The average hybridisation values from the number of determinations listed are normalised to the actual final extent of hybridisation obtained to baboon (*P. cynocephalus*) cellular DNA (86–100%). Cycled viral probes (see Fig. 2) and uncycled probes yielded comparable hybridisation values to the various species when normalised to the homologous system. Some baboon viral probes prepared using virus produced by human cells, however, gave artefactually high values with human cellular DNA. The standard deviation of all the data is shown in the next column.

‡The values obtained after hybridising the baboon viral probe to the primate cellular DNAs are shown; the average hybridisation value obtained with non-primate mammalian DNA has been subtracted. Non-mammalian DNA (salmon sperm) yields an even lower hybridisation value than that obtained for the non-primate mammals; this value has not been included in the average.

§The extent of hybridisation between the ³H-DNA probe prepared from the baboon type C virus and the cellular DNAs was detected on hydroxyapatite¹. The percentage hybridisation values shown are normalised values from four to seven experiments; the final extent of hybridisation to baboon cellular DNA varied between 74–88%. DNA–DNA hybridisations were performed at 56 °C in 0.025 ml reaction mixtures containing 0.01 M Tris, pH 7.4, 0.50 M NaCl, 2 × 10⁻³ M EDTA, 0.05% SDS, 2,000 c.p.m. of ³H-baboon type C DNA probe, and 0.1 mg of cellular DNA.

Table 4 Differences in extent of genomic alteration between African and Asian Old World monkey and ape virogenes

Table 1. Differences in extent of genomic alteration between African and Asian Old World monkeys and apes (virogenes)							
Primate	African origin			Primate	Asian origin		
	$\Delta T_m R$ (°C)* cellular DNA	No. of species	% Hybrid† virogene DNA		$\Delta T_m R$ (°C)* cellular DNA	No. of species	% Hybrid† virogene DNA
Old World monkeys							
			Cercopithecinae				
Mandrill	2.1	1	66	Celebes Ape	2.3	1	27
Mangabey	2.6	2	48, 51	Macques	2.5–2.9	4	25–28
Colobinae							
Colobus	6.5	1	24	Langur	6.4	1	5
Apes							
				Gibbon	9.0	1	3
Gorilla	9.5	1	10	Siamang	8.9	1	3
Chimpanzee	9.3	1	9	Orang-utan	8.9	1	3
Man	9.2	1	3	Man	9.2	1	3

*The $\Delta T_m R$ (°C) of hybrids formed between the cellular DNA of the primate species and ³H-non-repetitive baboon cellular DNA is shown. These values are from the data listed in Table 1.

†The normalised final percentage of hybridisation of primate cellular DNA to the baboon viral DNA probe is listed. These values are from the data shown in Fig. 2 and Table 3. By varying the S₁ nuclease incubation conditions a higher final extent of hybridisation to the various primate DNAs can be obtained. For example, by increasing the monovalent cation concentration from 0.15 M to 0.30 M and by decreasing the temperature of the S₁ nuclease incubation from 45 °C to 37 °C, the final extent of hybridisation of the baboon viral probe to mangabey DNA increases from 50% to 85%, and the value to rhesus and other macaque cellular DNAs increases from 25% to approximately 60%. The substantial difference between the various African and Asian primate DNAs remains, however.

The hybridisation data obtained with non-repetitive cellular DNA group the primates in terms of their phylogenetic proximity to the baboon (Fig. 3). The data obtained with the endogenous type C virogenes sequences show that the type C virogenes of the Asian primates hybridise less to the baboon probe than do the virogenes of their African counterparts. Thus, with the endogenous primate type C viral gene sequences (although not with the overall cellular DNA), we are able to discriminate quite clearly between Asian and African primates. Among the apes, the gibbon and orang-utan clearly stand out as Asian. Within the ape subfamily Hominae (man, chimpanzee and gorilla¹⁷), *Homo sapiens* stands out as the only non-African representative. We therefore propose an Asian origin for man, with a long history in that region before our species migrated all over the world.

An alternative interpretation of the data would be that man is unusual among primates in having a deletion (or series of deletions) in his virogenes sequences that makes its properties coincide with those of the orang-utan, gibbon and siamang. A partial deletion hypothesis in man, however, does not account for the marked differences in the extent of genomic alteration between the Asian and the African Cercopithecinae or Colobinae virogenes when tested with baboon viral DNA transcripts.

The results presented here show that the extent of nucleic acid dissimilarity between the primate virogenes depends on two factors: (1) the taxonomic distance of the species from *Papio*, and (2) the geographical habitat of the species. A comparison of baboon and mangabey type C virogenes, which have evolved together in Africa, and of baboon and macaque virogenes, where the species have been physically separated from each other for several million years, reveals that the differences in their virogenes sequences result from the effect of one environment as opposed to another. Thus the differences in thermal stability and final extent of hybridisation in this particular set of mammalian genes is influenced by environmental factors. The ubiquitous presence of endogenous type C virogenes among anthropoid primates and their evolutionary persistence for at least 30–40 Myr suggest that such genes provide functions with a selective advantage to the species possessing them. Thus, some functions of the type C virogenes are acted on by the environment in such a way as to select for certain genetic sequences and against others.

Type C viruses can be isolated readily from four different

species of baboons (*P. cynocephalus*, *P. anubis*, *P. papio*, and *P. hamadryas*) and from the closely related genus, *Theropithecus*^{18,19,24,25}, all of which inhabit Africa. Extensive attempts to isolate infectious type C viruses from other anthropoid primates using the same techniques that have led to the isolation of the baboon and gelada viruses, have so far been unsuccessful²⁶. The action of selective environmental pressure presupposes that the virogenes are expressed during the lifetime of the animal and, further, that they affect the survival or the reproductive capacity of individuals of the species. The primate species from which infectious viruses have not yet been isolated must therefore express at least some virogenes products on which the environment may act. Resistance to infection by exogenous type C viruses might be a critical selective factor; baboons range throughout Africa and release a virus that might be potentially damaging to recipient species. The endogenous domestic cat virus RD-114 has been postulated to have originated from an endogenous Old World monkey type C virus horizontally transmitted to an ancestor of the domestic cat²². This suggests the possibility that ancestors of the baboon may have been releasing infectious virus particles for several million years. The selective pressure on the other primates to maintain their related virogenes and so protect themselves against viral infection would be continuous and strong. Endogenous type C virogenes have previously been shown to restrict the replication of highly related viruses in the same cells²⁶. The African primates may well have had to conserve a greater degree of nucleic acid sequence homology relative to the baboon type C virus in order to restrict the replication of this virus in their cells. Those portions of the virogenes most directly involved in controlling virus spread from animal to animal would be expected to show the most extreme environmental effect.

Asian primates, which would not be exposed to the baboon endogenous virus, would be under less selective pressure to maintain cellular nucleic acid sequences similar to those present in the baboon type C viruses; their virogenes, then, would be free to accumulate genomic alterations at an apparently faster rate relative to baboon virogenes sequences. Rhesus monkey cells, for example, are extremely permissive for the replication of baboon type C viruses, whereas baboon, gelada and mangabey cells restrict baboon virus replication. Man, postulated here to be an Asian primate that has only recently migrated into Africa, would, therefore, be expected to be susceptible to infection by baboon type C virus. Human cell

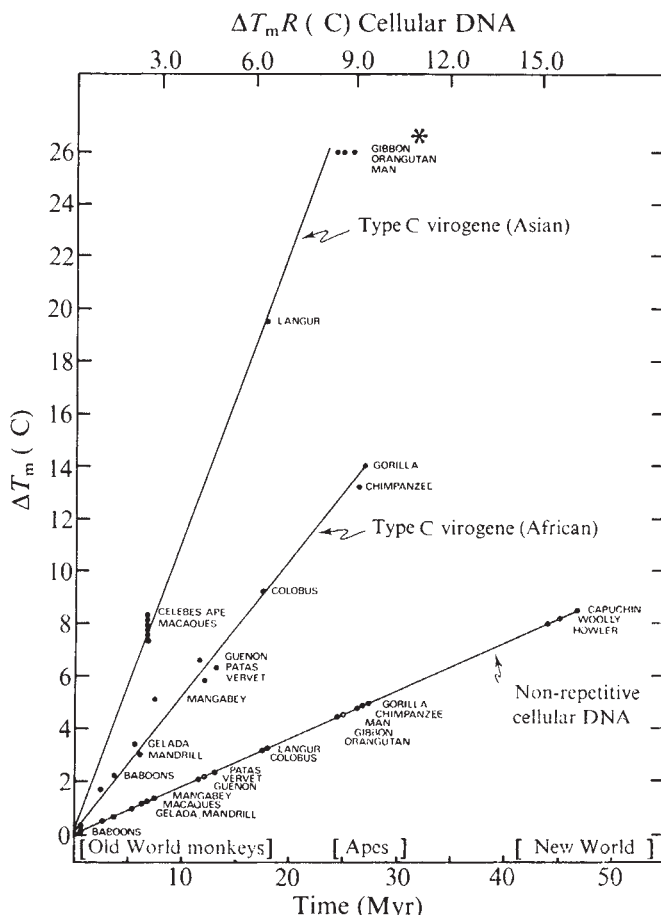


Fig. 3 Relationship among primate cellular DNAs as determined by the extent of their homology to baboon non-repetitive cellular DNA and to the baboon type C viral DNA probe. The genetic distance from the baboon (*P. cynocephalus*) of various primate species as determined by the thermal stability (ΔT_m) of hybrids formed between their cellular DNA and non-repetitive baboon DNA is shown in the lower curve. The thermal stabilities of the virogene sequences of all the African primates tested fall on the middle curve and those of all the Asian primates and also of man fall on the upper curve. No direct comparison can be made between the rate of base-pair changes in the cellular and viral nucleic acid sequences since the latter are present in 10–20 copies per haploid genome⁴⁴ whereas the cellular gene sequences are predominantly present in only one copy⁷.

*Accurate T_m values for the hybridisation of gibbon, orangutan and human cellular DNA to the baboon viral probe have not been determined experimentally; they are extrapolated from the data shown in Fig. 2. Nevertheless, these T_m values are lower than those obtained to gorilla and chimpanzee DNA.

cultures, in fact, have been shown to be highly susceptible to infection by various isolates of the baboon type C virus^{18,19,25}.

The rhesus monkey produces endogenous type C viruses that can be seen by electron microscopy²⁷, and expresses type C viral p30 antigen and reverse transcriptase^{28,29}, but so far, no infectious virus has been isolated. Such a virus would allow us to study Asian primate virogenes and perhaps determine whether they too are more closely related to one another than they are to the virogenes of African primates.

Possible implications for the origin of man

Because of man's close morphological and anatomical resemblance to the African apes, and because most of the recent palaeontological discoveries have been made in Africa, many investigators have proposed an African origin for our species. The results presented here lead to a conclusion that is not consistent with the palaeontological studies which place the

major developmental changes that have led to modern man as occurring in Africa; rather, they suggest to us that man has originated outside Africa.

The time of divergence of the hominid stock from the apes has variously been estimated at between 4 and 20 Myr; our data indicate a divergence time of approximately 12 Myr. *Ramapithecus* and *Kenyanthropus*, which have been dated by potassium-argon at 12–14 Myr were found in India and in East Africa^{30–32}. They seem, according to our data, to precede or perhaps to be contemporary with the man-chimpanzee-gorilla ancestor. There then follows a period of about 10 Myr, virtually all of the Pliocene, where fossil finds have been few and have not been extensively characterised. Between 1 and 3 Myr, fossils that appear to be hominids have again been found both in Africa and Asia. Our data suggest that man's direct ancestors probably spent most if not all of the Pliocene in Asia. The older Australopithecines found in Africa, though clearly hominids, were probably, therefore, not in the main lineage to man, but rather, unsuccessful offshoots whose progeny have not endured to the present³³. The data do not preclude a more recent migration, perhaps in the last 2–3 Myr, of hominids into Africa. The nucleic acid sequence changes we are measuring are the result of millions of years of evolution. The relatively recent migration, or return, of man's ancestors to Africa does not seem to have reversed the long evolutionary history of man's type C virogenes while in Asia. The much more recent migrations by modern man all over the world and his divergence in the last hundred thousand years into the various races are also events which are too recent to be detected with these techniques^{34,35}.

Of all the primates, the hominids have been the most successful at adapting to a terrestrial life and have migrated all over the world; their geographical origin, therefore, is more obscure than that of the other primates. The major selective pressures that influence the evolutionary history of a species are recorded and retained in its genetic material. The baboon viral gene sequences have allowed us to distinguish clearly between Asian and African primates and to study the imprint of this environmental selective pressure on primate cellular DNA. The data obtained with the primate virogenes suggest that, during the Pliocene, man's ancestors were developing in Asia.

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Rare earth, Fe and Ti variations along the Galapagos spreading centre, and their relationship to the Galapagos mantle plume

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Rare earths, Fe and Ti concentrations in basalts dredged along the Galapagos spreading centre reveal that a juvenile mantle plume is welling up beneath the Galapagos Islands and mixing with the depleted asthenosphere to the north-east and north-west beneath the ridge. Unusually extensive shallow-depth, crystal fractionation is also apparent on the ridge between 85 and 87°W. The 'magnetic telechemistry' hypothesis is further clarified.

THE plate tectonic theory provides a simple and unified explanation for surface geological features such as mountain ranges, trenches, island arcs and mid-ocean ridges, and describes their kinematic relationships. The theory does not, however, adequately explain shallow oceanic platforms such as those found around the Azores, Hawaii, Iceland, and the Galapagos Islands.

These unusually extensive outpourings of lava have been attributed to fixed hot spots¹ and mantle plumes². Vogt has suggested³ that flows of asthenospheric partial melts associated with mantle plumes could be monitored for hot spots located near the mid-ocean ridge, and that the flow is probably concentrated in a pipe-like region below the spreading axis; transform faults would tend to dam such sub-axial flow^{4,5}.

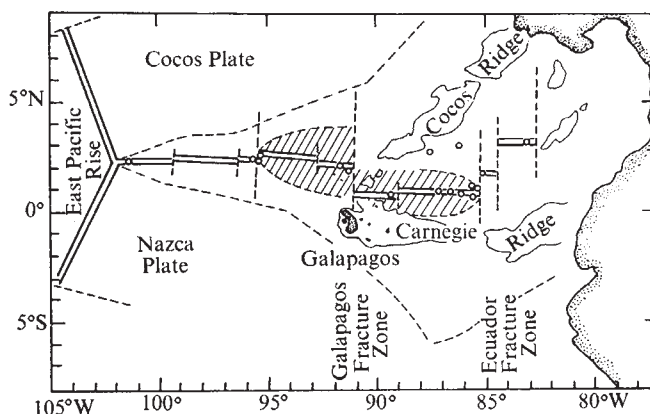
Schilling^{4,6} has mapped large-scale chemical gradients from basalts dredged from the axis of the Mid-Atlantic Ridge away from Iceland or the Azores, and attributed the gradients to binary mixing, at shallow depth beneath the ridge, of two geochemically distinct mantle slush components: namely a mantle plume enriched in light rare earths (RE) and radiogenic Sr and Pb isotopes, and a widespread asthenosphere depleted in light RE and low Sr and Pb isotopic ratios^{4,6–10} (subsequently referred to as depleted low velocity layer—DLVL).

We report here new geochemical data for the Galapagos spreading centre and its associated platform (Fig. 1). The area contains most of the typical elements of the Iceland

type of 'hot spot'¹¹. The Galapagos Archipelago is mainly built of tholeiitic lava of 'plume'-derived or 'blob'-derived composition, judging from their RE patterns^{12–14}. The Cocos and Carnegie aseismic ridges branch from the platform, and the Galapagos spreading centre shoals where it passes north of the platform (Figs 1 and 2). No regional free-air gravity high, relative to the figure of hydrostatic equilibrium, has been observed over the region, however. Unusually high magnetic anomaly amplitudes have also been noted over the crest of the Galapagos spreading centre within 5° of the platform (Fig. 1).

Vogt and Johnson¹⁶ have attributed the large magnetic anomaly to an oceanic crust rich in Fe and Ti derived from a mantle plume welling up beneath the Galapagos Islands and flowing outwards at shallow depth; Anderson, *et al.*¹⁷ also suggested Fe and Ti enrichment, but suggested

Fig. 1 Location of the dredge hauls studied (circles) along the Galapagos spreading centre (double lines), and their relationships to the high magnetic amplitude region (hatched area) and major tectonic elements, namely: the Cocos and Carnegie aseismic ridges; the Galapagos Islands (blackened); fracture zones (heavier vertical dashed lines); the Galapagos Gore (light dashed line)^{11,32}.



that it is caused by a large 'melting anomaly' centred about the Galapagos Archipelago with exceptionally intense, shallow-depth, fractional crystallisation of plagioclase feldspar and olivine from a normal, light RE-depleted mid-ocean ridge basaltic melt. Relative RE patterns of the Fe-rich residual liquids produced by the latter mechanism, although enriched as a whole, would remain depleted in light RE elements, and should develop an anomalous Eu depletion (proportional to the extent of plagioclase removal and anorthite content)^{13,18}, whereas mantle plume basalts are commonly enriched in light RE elements, and generally show no Eu anomaly. The RE distinction can thus serve as a simple test of the two mechanisms and origins proposed for the region of high magnetic amplitude over the Galapagos spreading centre.

Results from this pilot study show the effect of local Fe enrichment caused by the shallow plagioclase-clinopyroxene and olivine crystal fractionation of DLVL-derived basalts. They also indicate mantle-plume mixing along the ridge segment nearest to the Galapagos Archipelago. Extensive shallow crystal fractionation seems to be limited to 85–87°W along a segment of the ridge apparently not affected by the Galapagos mantle plume.

Results

Locations of the dredged rocks are shown in Fig. 1. The RE, FeO* and TiO₂ concentrations, and FeO*/FeO*+MgO ratios of these rocks are given in Table 1. Major element analyses and magnetic properties of rocks from stations T6, HFD-1, D-7 and D-17 have already been reported by Anderson, *et al.*¹⁷. The complete major element analyses of rocks from USNS De Steiguer dredge hauls are presented in Table 2. These samples were obtained on either side of the boundary delineating the high magnetic anomaly province, to define the sharpness of the transition. Cocotow dredged rocks were obtained with R. V. Melville, and their major element chemistry will be reported elsewhere (J. W. Hawkins, unpublished). The chemistry of basalt RC-10-56 has already been reported¹⁹. Highly differentiated and atypical oceanic volcanic rocks were also dredged north of

Fig. 2 *a*, La/Sm concentration ratio (normalised to chondrites); *b*, TiO₂ weight percentages; *c*, FeO*; *d*, variations along the ridge axis of the central magnetic anomaly; *e*, water depth. Later data from Anderson, *et al.*¹⁷. For comparison, dotted-dashed lines are averages of 13 dredged basalts from the East Pacific Rise between 0 and 19°S (ref. 22), (FeO* = 9.2 ± 0.5 weight %; TiO₂ = 1.41 ± 0.22 weight %; and [La/Sm] = 0.60 ± 0.12). The averages exclude the three ferrobasalts also reported for this normal EPR segment. The error bars attached to the averages are for 1 s.d. Open symbols, dredge stations off the ridge axis; solid symbols, stations on the axis.

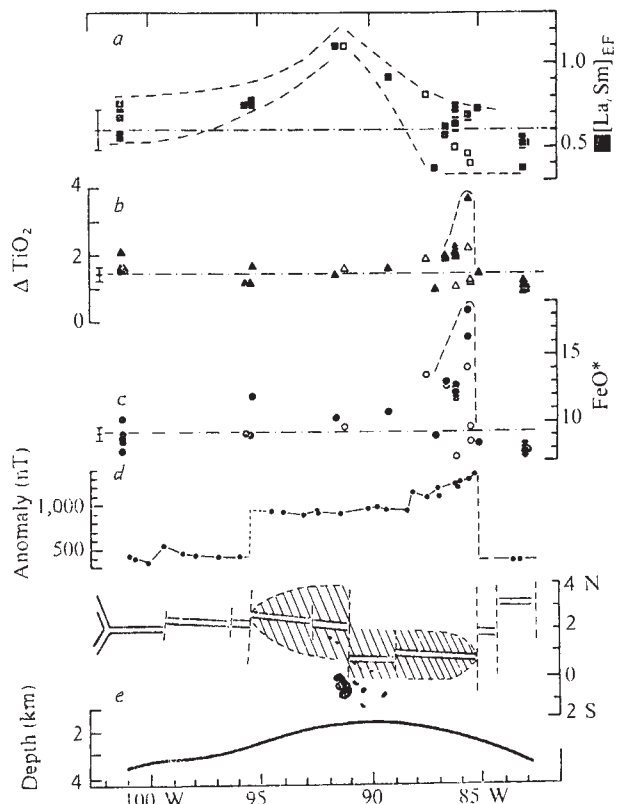


Table 1 Abundances of rare earths (p.p.m.), FeO* and TiO₂ (weight %) of dredged basalts from the Galapagos spreading centre†

Cruise	Station	Latitude	Longitude	Depth (m)	La	Ce	Nd	Sm	Eu	Tb	Tm	Yb	Lu	[La/Sm] _{EF}	FeO*	TiO ₂	FeO*/FeO*+MgO
Southtow 7	D-7-1	3°12.0'N	83°02.4'W	2,502	1.3			1.8	0.64			2.0	0.32	0.52	8.30	0.87	0.525
	D-7-2	3°12.0'N	83°02.4'W	2,502	1.8			2.3	0.88			1.8	0.35	0.55	7.88	1.03	0.476
	D-7-3	3°12.0'N	83°02.4'W	2,502	1.1			2.1	0.78	0.51		2.3	0.34	0.37	7.59	1.02	0.478
	D-7-4	3°12.0'N	83°02.4'W	2,502	1.9			2.6	0.96	0.69		2.5	0.32	0.52	7.60	1.10	0.481
	D-7-5	3°12.0'N	83°02.4'W	2,502	2.1			2.9	1.1	0.77		3.1	0.37	0.51	7.71	1.23	0.557
	D-17-1	0°49.2'N	86°07.8'W	2,600	5.3			5.8	1.8	1.4		4.3	0.81	0.65	12.22	2.05	0.671
	D-17-2	0°42.9'N	86°07.8'W	2,600	7.0			6.5	2.0	1.4		6.0	0.92	0.75	12.39	2.14	0.683
	D-17-3	0°49.2'N	86°07.8'W	2,600	4.9			5.3	1.7			5.6	0.79	0.65	11.82	2.04	0.653
	D-17-4	0°49.2'N	86°07.8'W	2,600	4.8			5.2	1.7	1.3		6.3	0.80	0.63	11.90	2.01	0.653
	D-17-5	0°49.2'N	86°07.8'W	2,600	6.8			6.6	2.0	1.6		5.5	0.88	0.72	12.38	2.15	0.706
	HFD-1	3°00.0'N	86°00.10'W		1.6			2.2	0.87	0.44		2.3	0.35	0.49	7.46	1.00	0.470
Bartlett	T6-378	2°12.0'N	101°25'W	3,300–4,790	4.3			5.4	1.8			4.3	0.85	0.56	10.32	2.07	0.618
	T6-269	2°12.0'N	101°25'W	3,300–4,790	3.4			4.1	1.5			3.6	0.50	0.58	7.83	1.54	0.556
	T6-384	2°12.0'N	101°25'W	3,300–4,790	3.3			3.3	1.3			3.2	0.43	0.68	8.91	1.55	0.560
	T6-285	2°12.0'N	101°25'W	3,300–4,790	3.5			3.5	1.3			3.2	0.46	0.71	8.76	1.54	0.557
	T6-372	2°12.0'N	101°25'W	3,300–4,790	4.5			4.2	1.3			3.3	0.50	0.76	8.81	1.56	0.560
De Steiguer 41	D1A	0°42.6'N	85°30.00'W	2,377	9.8	31.9	29.0	10.2	2.9	2.6	1.8	10.7	1.5	0.68	18.36	3.62	0.809
	D1B	0°42.6'N	85°30.00'W	2,377	7.2	22.2	20.9	7.4	2.5	2.0		8.1	1.2	0.68	16.34		
	D2A	1°44.4'N	85°07.2'W	2,798	3.8	13.4	10.3	3.6	1.3	0.86	0.51	3.2	0.44	0.73	8.47	1.41	0.495
	D3A	1°02.3'N	85°25.4'W	1,554	1.5	7.1	6.1	2.6	1.0	0.79	0.56	3.1	0.45	0.39	9.59	1.23	0.608
	D3B	1°02.3'N	85°25.4'W	1,554	1.6	5.1	6.6	2.8	1.0	0.79	0.50	3.0	0.46	0.40	8.53	1.21	0.565
	D4	1°08.6'N	85°30.00'W	2,926	3.5	13.6	13.7	5.4	1.6	1.4	0.96	5.4	0.79	0.46	13.98	2.16	0.710
	D5	2°26.4'N	95°37.2'W	2,743	2.8	8.6		2.6	0.93	0.66	0.40	2.6	0.38	0.75	9.10	1.10	0.503
	D7A	2°25.2'N	95°24.6'W	2,103	2.8	9.1	6.7	2.7	0.96	0.72	0.48	2.7	0.38	0.75	9.04	1.13	0.527
	D8A	2°36.0'N	95°19.8'W	3,109	4.5	13.5	10.7	4.0	1.4	1.1	0.81	4.4	0.61	0.78	11.96	1.62	0.614
Cocotow 4	6D-1	0°51.1'N	86°32.8'W	2,330–2,432	4.6	16.4	13.5	5.2	1.7	1.3	0.94	5.4	0.80	0.62	12.77	1.89	0.654
	6D-2	0°51.1'N	86°32.8'W	2,330–2,432	3.8	12.9	11.8	4.7	1.7	1.3		5.1	0.75	0.57	12.95	1.87	0.667
	7D-1	0°51.8'N	87°05.1'W	2,385–3,230	1.1	5.8	4.8	2.1	0.90	0.71	0.50	2.7	0.32	0.37	8.98	0.94	0.504
	8D-1	2°38.4'N	87°29.1'W	2,347–3,170	5.5	17.5	12.0	4.8	1.7	1.2	0.84	4.7	0.71	0.81	13.53	1.86	0.686
	9D-1	0°46.7'N	89°13.7'W	1,777–1,845	4.9	13.3		3.7	1.4	0.96	0.63	3.0	0.45	0.91	10.72	1.54	0.592
	10D-1	1°59.9'N	91°36.6'W	1,981–2,050	5.1	13.8	10.9	3.4	1.3	0.84	0.66	3.2	0.52	1.1	10.29	1.34	0.575
RC-10-56		1°48.6'N	91°15.3'W	2,082	5.8	19.1	13.9	3.6	1.4	0.88	0.51	3.1	0.45	1.1	9.62	1.60	0.568
JB-1 Split No. 8†					39.4 ± 6.8%	75.6 ± 8.1%	29.5 ± 9.1%	5.4 ± 9.2%	1.6 ± 6.2%	1.2 ± 8.3%		1.9 ± 21.0%	0.30 ± 3.3%				

† RE patterns were obtained using instrumental neutron activation analysis (R. Kingsley); FeO* (total iron as FeO), TiO₂, and MgO by wet chemical analyses (R. Evans).
‡ Basalt standard JB-1, average of 5 replicate analyses and 1 standard deviation (%).

Table 2 Major element analyses of dredged basalts from De Steiguer Cruise 41 (Galapagos spreading centre)*

Sample No.	D1A	D2A	D3A	D3B	D4	D5	D7A	D8A
SiO ₂	48.43	49.55	50.95	51.39	50.49	49.76	50.15	50.23
Al ₂ O ₃	11.51	16.58	15.84	16.11	13.14	15.71	15.87	14.22
Fe ₂ O ₃	3.61	1.15	2.70	1.92	4.03	1.17	1.44	1.74
FeO	15.11	7.44	7.16	6.80	10.35	8.05	7.74	10.39
MgO	4.33	8.65	6.18	6.57	5.72	9.00	8.12	7.52
CaO	8.95	11.57	12.46	12.61	10.38	12.09	12.27	11.06
Na ₂ O	2.66	2.77	2.33	2.33	2.55	2.26	2.22	2.34
K ₂ O	0.24	0.08	0.15	0.12	0.06	0.07	0.11	0.14
H ₂ O ⁺	0.91	0.21	0.49	0.45	0.33	0.21	0.38	0.36
H ₂ O ⁻	0.17	0.04	0.29	0.21	0.38	0.09	0.14	0.09
TiO ₂	3.62	1.41	1.23	1.21	2.16	1.10	1.13	1.62
P ₂ O ₅	0.32	0.21	0.11	0.11	0.22	0.11	0.11	0.19
MnO	0.26	0.15	0.17	0.16	0.24	0.16	0.17	0.20
Total	100.12	99.81	100.06	99.99	100.05	99.78	99.85	100.10

*Analyst, R. Evans

the axis near 95°W and on the 85°W fracture zone, but are not considered here.

Petrographically, the basalts studied for RE show quenched textures typical of pillow basalts, and range from aphyric to plagioclase or, more rarely, olivine, porphyritic basalts. The new De Steiguer samples are classified as tholeiitic basalts according to Yoder and Tilley's²⁰ normative scheme (Fig. 3). They range from olivine normative to mildly quartz normative; and follow a Skaergaard trend on the AFM diagram (Fig. 3), with extreme iron enrichment reaching 18% FeO* for station D1, near 85°30'W. Cocotow rocks (J. W. Hawkins, unpublished), Southtow basalts¹⁷ and RC10-56¹⁹ fall on the same iron fractionation trend.

Basalts from stations Southtow D-17 and Batlett T6 tend to be alkali rich relative to common mid-ocean ridge basalts²¹. Since Fe₂O₃/FeO proportions were not determined by Anderson, *et al.*¹⁷, Southtow D-7 and HFD-1 are not directly comparable with the De Steiguer samples. Anderson, *et al.*'s basalts¹⁷ are, however, close to the Di normative join if Fe₂O₃ is assumed to be 1.5% (ref. 18). It is possible that the high alkali content of station T6 is attributable to alteration by sea water, and is not an intrinsic characteristic of melts erupted near the Hess Depression. On the other hand, the rare earth patterns of station T6 samples are

depleted in light RE, and are typical of other tholeiitic basalts erupted along normal ridge segments (Fig. 4).

RE variations along the ridge

Figures 2 and 4 show that all basalts located beyond 450 km west of east of the Galapagos Fracture Zone are depleted in light RE elements, including those from station T6 near the triple junction. Judging from such evidence¹⁰, these basalts seem to have been derived from the light RE-depleted asthenosphere source, whether they were erupted within or outside the high magnetic amplitude region.

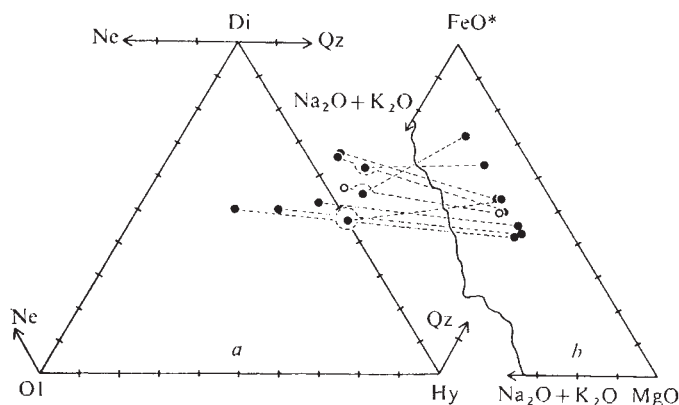
In contrast, ridge basalts enriched in light RE elements are found only near and just north of the Galapagos Archipelago (Figs 1 and 4). Some 190 km east of 91°W, an intermediate flat RE pattern is found. Yet, normatively, basalts from these later three stations are indistinguishable from the light RE-depleted basalts found further out from the Galapagos Islands (Fig. 3). Tholeiitic basalts with such light RE enrichment suggest mantle plume derivation^{4,6}. The degree of light RE enrichment in these three stations is, however, too low relative to tholeiitic basalts from the Galapagos Archipelago to be 100% derived from the mantle plume^{12,13}.

It seems that the distribution of RE patterns along the ridge show no simple relationship with magnetic anomaly amplitudes. Basalts with both light RE-enriched and light RE-depleted patterns occur within the high magnetic amplitude region; and light RE-depleted patterns occur both within and outside the high magnetic zone as well. Judging from the RE patterns so far available, mantle plume mixing with DLVL material seems to be limited to a radius of approximately 450 km from the centre of the Galapagos Archipelago, and not to the entire length of the high magnetic amplitude zone.

Chemical correlations along the ridge axis

The variations of iron, titanium and light RE fractionation in basalts erupted along the Galapagos spreading centre are shown in Fig. 2 in relation to variations of the magnetic anomaly intensity, and ridge elevation. The chemical variations are compared with averages obtained along the East Pacific Rise (EPR) between 0 and 20°S. This part of the EPR seems geochemically typical of normal mid-ocean ridge segments^{10,22}, and it is regionally close to the Galapagos region. Figure 2 shows that outside the high magnetic anomaly region of the Galapagos spreading centre, the Fe content of the basalts closely straddles the average value found for the EPR; with the exception of somewhat lower values near 83°W. On the other hand, within the magnetic

Fig. 3 a, Quartz - diopside - hypersthene - olivine - nepheline normative diagram of De Steiguer-dredged basalts: solid circles, light RE-depleted; open circles, RC-10-56 light RE-enriched basalt. No relationship is apparent between RE patterns and normative mineralogy. b, FeO* (total iron as FeO), Na₂O + K₂O, MgO diagram for the same samples. Bulk chemistry data for De Steiguer samples are given in Table 2, and for RC-10-56 in ref. 19. Relationship between Fe/Mg variation and normative mineralogy is indicated by tie lines between the two diagrams.



anomaly zone, there is a tendency for the Fe content to be somewhat higher than the EPR average; whereas the TiO_2 content is not greatly different from that in samples from outside the zone, and is generally close to that of the EPR. Finally, between 85 and 87°W unusually large FeO^* and TiO_2 enrichments occur corresponding to the area of highest magnetic anomaly intensities. A relationship between magnetic intensity and Fe enrichment thus seems valid; but so far no obvious relationship is apparent with Ti outside the 85–87°W region. Further, it needs to be noted that low FeO^* and TiO_2 values occur together with the high values in the area between 85 and 87°W. There also seems to be no systematic variation in Fe and Ti enrichment with distance or age across the ridge in the 85–87°W area.

Basalts from De Steiguer station D1 located on the ridge axis (~0 Myr), are the most Fe enriched (18% FeO^*). Basalts from station 3D, dredged between magnetic anomalies 1 and 2 (~1.3 Myr), are close to the EPR average ($9.2\% \pm 0.5 \text{ FeO}^*$), and are thus normal. Basalts from station 4D, the northernmost station (~1.7 Myr), have intermediate FeO^* contents (~15% FeO^*). Both stations 4D and 3D were near the edge of the high amplitude zone. These observations suggest that the correlation between magnetic intensity and Fe–Ti content is not simple, as pointed out by Watkins (and Vogt and Johnson)²³. Among other things, one would also have to know the relative volumes of iron-rich to normal lavas, to establish further a telechemical relationship between Fe (and perhaps Ti) enrichments and magnetic anomaly intensity.

The variation of the La/Sm ratio along the ridge shows a progressive decrease with distance (or depth) from the Galapagos platform; however, the ratio does not correlate directly with FeO^* or TiO_2 content (Fig. 2). Thus, Vogt and Johnson's¹⁸ idea of correlating Fe and Ti enrichments with $[\text{La}/\text{Sm}]_{\text{EF}}$ for the Galapagos spreading centre (and the Iceland–Reykjanes Ridge as well; J.-G. S., unpublished), seems to be invalid. The reason is that FeO^* , TiO_2 and large ionic lithophile (LIL) element concentrations and their ratios in basalts, are not only functions of the type of mineral fractionation involved during partial melting in the mantle, fractional crystallisation during magma ascent, or stagnation in magma chambers, but also of the content of the mantle source(s) from which basalts are derived. No simple relationship can theoretically be predicted which would be of general application and independent of the geological processes and mantle sources involved. That should not, however, detract from the fact that the La/Sm ratio remains a reliable source indicator when dealing with tholeiitic basalts. Schilling¹⁰ has shown that the La/Sm ratio of a tholeiitic basalt produced by some 20% or greater degree of partial melting, is practically identical to that of its mantle source, to within 20% or less. The ratio varies by more than a factor of two along the Galapagos spreading centre, and is independent of the Fe or Ti contents. The apparent decrease in the La/Sm ratio outwards from the Galapagos, suggests mantle plume mixing with DLVL material within a radius of approximately 450 km from the centre of the Archipelago. Even extensive shallow-depth fractional crystallisation of the olivine-gabbro type²⁴ cannot produce the light RE enrichment found in tholeiites occurring near the Galapagos Islands.

Fe enrichments of DLVL-derived basalts between 85 and 87°W

Figure 4 shows that the extent of RE enrichment and Eu depletion in basalts erupted between 85 and 87°W correlate closely with the Fe content of Galapagos lavas, although the degree of light RE fractionation (that is, the La/Sm ratio) remains rather insensitive to the Fe enrichment mechanism. We have modelled quantitatively such Fe and RE enrichments by fractional crystallisation. First, we have

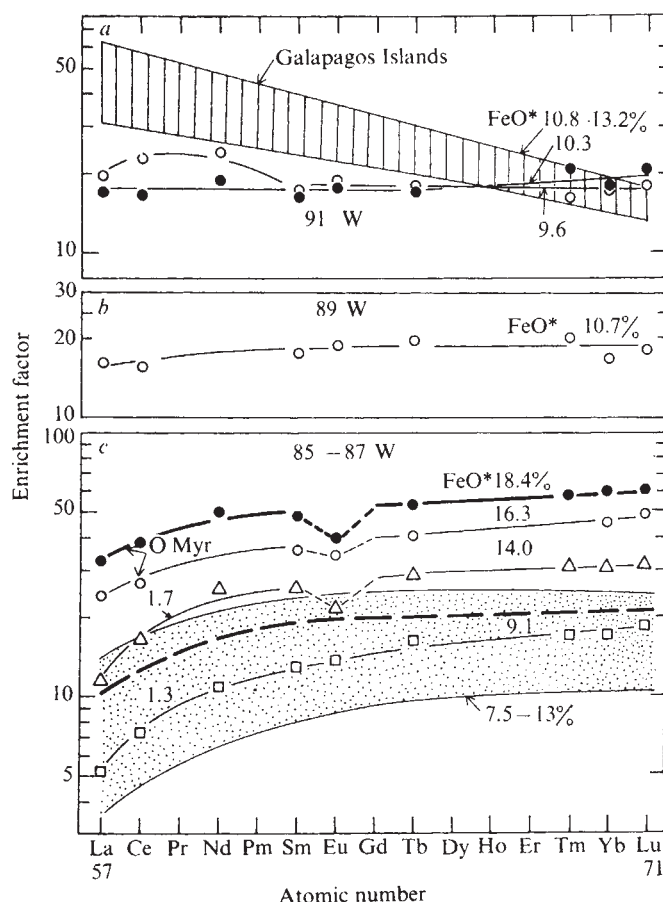


Fig. 4 Chondrite normalised rare earth patterns of dredged basalts from the Galapagos spreading centre. *a* and *b*, Basalts located within a radius of 450 km from the archipelago. Hatched area, the field occupied by four Galapagos Island tholeiites studied petrochemically by McBirney and Williams¹². Isabela (E-132), Baltra (E-28), Marchena (E-15) and the north-westernmost island of the archipelago, Darwin Island (E-35); $[\text{La}/\text{Sm}]_{\text{EF}}$ ratios are 1.67, 1.45, 1.15 and 1.49; and FeO^* , 12.7, 13.2, 11.4 and 10.8 w%, respectively). Rare earth data from this laboratory (unpublished). *c*, Shaded area shows range obtained for basalts located outside a 450-km radius, east of 87°30'W and west of 95°W; all light RE depleted (see data in Table 1). De Steiguer stations located near the eastern end of the high magnetic amplitude region shown separately in relation to their FeO^* content and their estimated magnetic age: D-1A (solid circle); D-1B (open circle); D-4 (triangle); average of D-3A and B (square). Note good correlation between total RE content and FeO^* . Heavy dashed line is calculated model RE pattern of original melt discussed in text. RE and FeO^* data taken from Table 1.

tried to satisfy the major element chemistry of the basalts using Wright and Doherty's²⁵ linear programming technique which allows determination of the type and extent of mineral extract. We then tested such mineral extracts against RE patterns of these rocks using the Doerner–Hoskin's logarithmic law for describing the change of the RE concentrations during fractional crystallisation²⁶. In this process we found, as did Kay, *et al.*¹⁸, that the Fe enrichment trend is accompanied by a decrease in the MgO , CaO and Al_2O_3 contents, and an increase in the TiO_2 , P_2O_5 and, to a lesser extent, Na_2O contents; whereas the SiO_2 content stays practically constant, or decreases slightly. Contrary to the findings of Kay, *et al.*, however, we were not able to minimise SiO_2 residuals by extracting only olivine and plagioclase, but had also to extract a significant amount of clinopyroxene. Table 3 illustrates our point for an extreme case. This model shows that the most differentiated Fe-rich basalt, De Steiguer-D1, can be derived from a melt of the composition averaging Southtow D-7 by 75% fractional

crystallisation of a mixture composed of plagioclase (An 62), clinopyroxene (En 48.5, Fs 10, Wo 41.5) and olivine (Fo 55), in proportions 0.54, 0.37 and 0.09, respectively. The proportions are reasonably close to the ternary eutectic (0.435 An, 0.49 Di and 0.075 Fo) experimentally determined for the simplified basalt system diopside-forsterite-anorthite²⁷; thus adding some credibility to the model.

By inverting the problem, the RE pattern of the 'original melt' was calculated from the DS-D-1 residual melt RE pattern, using the crystallisation model, and the average crystal-melt RE partition coefficients listed in Table 3. The calculated RE pattern of the 'original melt' is shown in Fig. 4. It is light RE depleted and no longer shows any Eu anomaly, as expected. The level of overall RE pattern enrichment satisfactorily falls within the range occupied by other 'normal ridge basalts' so far studied along the Galapagos spreading centre.

source significantly enriched in light RE elements relative to the DLVL-source feeding normal segments of the Galapagos spreading centre.

Conclusions

Several points need to be emphasised on the basis of this pilot study.

- Both light RE-enriched and depleted types of RE patterns occur within the region of high magnetic anomaly amplitudes. Light RE-depleted patterns also occur both within and outside the zone of high magnetic anomaly amplitudes. Thus, no simple relationship is readily apparent between types of RE pattern and the intensity of magnetic anomaly amplitudes.
- The highest Fe and Ti enrichments occur where the magnetic anomaly amplitudes are also largest (between 85 and 87°W). The RE and major elements in these basalts

Table 3 Fractional crystallisation model*

Oxide	Model extraction						Rare earth partition coefficients†			
	Primary melt (St 7-D7)	Clinopyroxene	Plagioclase	Olivine	Differentiated melt (DS D1A)	Residuals	Element	K_{cpx}	K_{plag}	K_{ol}
SiO ₂	50.90	51.50	52.78	36.73	50.75	-0.07	La	0.180	0.172	0.0089
Al ₂ O ₃	16.38	4.09	30.20	0.00	12.07	-0.02	Ce	0.256	0.153	0.0090
FeO	7.60	5.66	0.00	31.92	15.84	-0.02	Sm	0.571	0.092	0.0105
MgO	8.04	17.28	0.00	31.37	4.54	0.00	Eu	0.610	0.522	0.0110
CaO	13.28	20.75	12.57	0.00	9.38	-0.01	Yb	0.560	0.0781	0.0230
Na ₂ O	2.50	0.22	4.47	0.00	2.79	0.27	Lu	0.540	0.0810	0.0270
K ₂ O	0.09	0.00	0.00	0.00	0.26	-0.07				
TiO ₂	1.04	0.36	0.00	0.00	3.80	0.02				
P ₂ O ₅	0.07	0.00	0.00	0.00	0.34	0.09				
MnO	0.16	0.19	0.00	0.00	0.28	-0.14				

Total mineral extract: 75%.
 Mineral extracts: 28% Cpx.; 40.5% Plag.; 6.5% Ol.
 Modal mineral extracts: 0.37 Cpx.; 0.54 Plag.; 0.09 Ol.
 Plag. = An 62. Oliv. = Fo 55. Cpx. = Wo 41.5; En 48.5; Fs 10.0

*Minimum residuals were obtained by arbitrarily disregarding Fe₂O₃ contents and recalculating the analyses to 100% on a water free basis.
 †K is defined as trace element concentration in crystal relative to the melt.

We conclude that the shallow-depth fractional crystallisation model satisfactorily explains the Fe-rich basalts and associated RE patterns occurring near 86°W. The shallow-depth crystallisation process seems to be dominated by extractions of plagioclase, and to a lesser extent of pyroxene and olivine, from melts originally derived from the DLVL source. The model is thus consistent with Anderson, *et al.*'s¹⁷ suggestion, but seems to be limited to the 85–87°W area.

Finally, we emphasise that even in the extreme case of some 18% FeO* enrichment apparently requiring some 70–80% extent of crystallisation, the RE patterns of such residual liquids have retained the light RE-depleted patterns of the original melt, characteristic of normal ridge segments, although they develop a noticeable Eu depletion relative to RE neighbours, and a higher overall level of enrichment. In contrast to the 85–87°W area, ferrobasalts¹² from James Island (FeO* = 12.4%) and Jervis Island (FeO* = 13.4%) are light RE enriched (La/Sm_{EF} = 1.53 and 2.03, respectively) and are comparable with other subaerial tholeiites from the archipelago (Fig. 4). Those considerations suggest that the light RE-enriched patterns found in the two basalts erupted along the Galapagos spreading centre just north of the archipelago, can hardly be the product of shallow-depth fractional crystallisation. The two basalts show no Eu depletion or overall enrichment of the relative RE patterns, nor do they show any FeO* enrichments. More likely, these later basalts were derived from a mantle

suggest unusually extensive, shallow-depth fractional crystallisation of the olivine gabbro type occasionally found along normal ridge segments^{10,18,22}. The crystallisation model based on the new De Steiguer samples is thus consistent with Anderson, *et al.*'s¹⁷ explanation for the high magnetic anomaly amplitude near 86°W.

● On the other hand, our preliminary data suggest that a shallow-depth olivine gabbro crystallisation mechanism, such as that found between 85 and 87°W, is apparently not the mechanism responsible for the high magnetic anomaly across the whole region, contrary to the suggestion of Anderson, *et al.*¹⁷ (see Fig. 2). That is evident from the fact that no overall RE enrichments and relative Eu depletions are found within this zone, nor is the Fe content of the basalts markedly and systematically higher, except between 85 and 87°W. Additional sampling is required to resolve the question of the high magnetic amplitude region. Mild Fe enrichments in the area cannot be ruled out as an explanation.

● The telechemical idea¹⁶ of correlating magnetic anomaly amplitudes directly to Fe and Ti content of basalts, remains a complex and open question. Similarly, the idea¹⁶ of using the intensity of magnetic amplitudes to infer Fe and Ti contents, and in turn an array of LIL elements (and by inference their ratios as well) and Pb and Sr radiogenic isotopes, has no general validity, since Fe and Ti enrichment may have several causes. For example, Fe and Ti enrich-

ments can be obtained from either a mantle depleted in light RE along normal ridge segments (for example, as at the Galapagos spreading centre near 86°W), or from a mantle with light RE-enriched patterns and relatively richer in Sr and Pb radiogenic isotopes (for example, in the mantle beneath Iceland and the northern part of the Reykjanes Ridge^{4,7,9}, and the Galapagos Islands¹²). Thus, the idea¹⁶ of using magnetic amplitudes to infer Fe and Ti contents to identify plume-derived oceanic crust, is ambiguous on this basis alone, and could be misleading.

● Basalts with light RE-depleted RE patterns, characterising 'normal ridge segments' are confined to east of 87°W, and west of 95°W. On this basis, the two segments of the Galapagos spreading centre situated between 95°W and 101°W (including the triple junction with the East Pacific Rise) and 87° to at least 83°W, are considered 'normal ridge segments'. Basalts erupted along these two segments seem to have been derived from the DLVL mantle source.

● So far, ridge basalts with RE patterns similar to those of light RE-enriched mantle plumes are confined to a radius 450 km from the centre of the Galapagos Archipelago. Maximum [La/Sm]_{EF} values (1.1) are found just north of the main islands, but these values seem to be diminished already by mixing with DLVL material, relative to tholeiitic basalts from the Galapagos Islands^{12,13}. The data are consistent with the presence of a mantle plume rising beneath the Galapagos platform. The sampling along the Galapagos spreading centre, however, is so far insufficient to decide whether the mantle plume flow in the asthenosphere occurs radially about the Galapagos platform; or preferentially along certain directions, such as generally northward towards the Galapagos spreading centre, or perhaps channelled along the major Galapagos Fracture Zone. Our data are also insufficient to infer whether the Galapagos mantle plume is, or is not, richer in Fe and Ti contents relative to the surrounding DLVL mantle source.

● Finally, the cause of unusually extensive shallow-depth fractional crystallisation of DLVL-derived basalts near 86°W, remains puzzling. Unusually extensive hydrothermal activity has been reported in the area^{29,30} compared with elsewhere on the Galapagos spreading centre³¹. It is con-

ceivable that unusually intense hydrothermal circulation might have been removing heat more efficiently from a magma chamber generated by spreading, thus enhancing locally fractional crystallisation before lava eruption on the sea floor. The model remains to be quantified in terms of the various rates involved.

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letters to nature

Transient X-ray source A0620—00 observed at 408 MHz

THE transient X-ray source A0620—00 reported by Elvis, *et al.*¹ was also detected as a transient radio source at a number of observatories^{2–5} at frequencies from 962 to 5,000 MHz. We report here the detection of the source at 408 MHz using the Molonglo radio telescope on August 20, 1975. Since the result is based on a single observation we shall discuss the steps taken to ensure that the detection is real.

The Molonglo telescope is a transit instrument with 11 simultaneous beams in declination covering a strip of sky 15° wide. Because of the large uncertainty in the position of the source available to us at the time, several nights were required to map the area. By the time we became aware of a reliable position for the source it was no longer detectable above the noise level.

A source of 0.3 Jy was observed on August 20, very close to the accurate position of the object identified with the X-ray source⁶. No such source was detected on a previous survey

record nor on records taken subsequent to the decay of the X-ray source. As the r.m.s. noise for a single observation is 0.04 Jy, the detection level of ~7 s.d. is well above the possibility of a random noise fluctuation. The 408-MHz position compared with that for the optical source is

optical 06 h 20 min 11.2 s —00°19'10"
408 MHz 06 h 20 min 11.6±0.6 s —00°18'18±20"

The right ascension agrees extremely well but the declination differs by 2.6 s.d. The probability of a radial discrepancy of this order is ~3%, so it is not highly significant on the assumption of noise errors alone.

Table 1 Flux density

Date	Flux density at the optical position (Jy)
December 29, 1973	—0.08
August 20, 1975	+0.28
September 6, 1975	+0.02
September 7, 1975	0.00
September 17, 1975	—0.03
December 28, 1975	—0.08

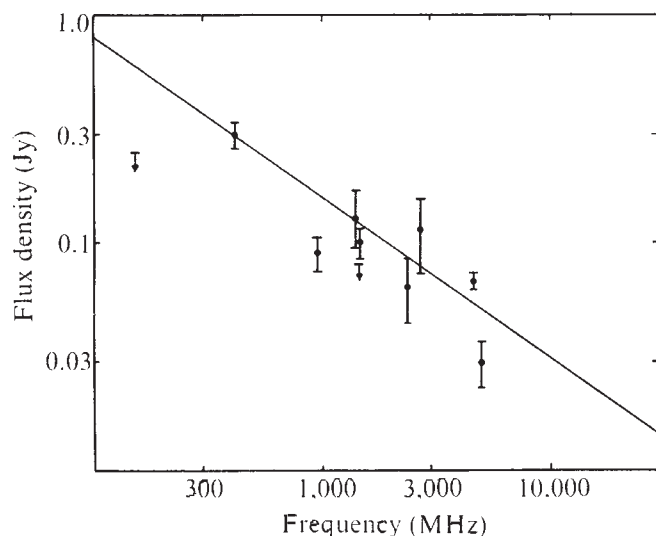


Fig. 1 The flux density of the radio source associated with the X-ray object A0620-00 plotted as a function of frequency. All points have been reduced to the common date of August 19.9, 1975 (UT). A line corresponding to a non-thermal spectrum (see text) is shown for comparison.

There remains the possibility of confusion with a weaker source or with a weak side lobe which may be either positive or negative. These effects are normally very small but in the galactic plane cannot be entirely ignored. We have therefore calculated the flux density at the optical position for all days on which this region of sky was observed (Table 1).

The estimated r.m.s. noise error on each of the above measurements is 0.04 Jy. The mean of all days other than August 20, 1975 is -0.03 ± 0.02 Jy. The relative flux density on August 20 is 0.31 ± 0.05 Jy which is highly significant and free of confusion. Based on the rate of decay of the source at higher frequencies the expected flux density at 408 MHz on September 6-7 would be <0.01 Jy and these have been considered as background points.

The flux density at 408 MHz is much larger than the higher frequency values. The available data are plotted in Fig. 1. The points have been reduced to the common epoch of August 19.9 UT using decay rates where available and using a time constant of 4.5 d for all other data.

The high flux density at 408 MHz is consistent with the suggestion of Owen *et al.*⁷ that the spectrum could be non-thermal. In fact a spectral index α ($S = S_0 \nu^\alpha$) of ~ -0.7 as shown in Fig. 1 fits the data quite well except that the points obtained with 3" resolution tend to fall somewhat below the line.

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Explanation for the very low Ga and Ge concentrations in some iron meteorite groups

THE Ge/Ni ratios in iron meteorites range from 10^{-2} to 10^{-7} atoms/atom (ref. 1). Gallium is strongly correlated with Ge, but the total range in Ga/Ni ratio is only about 2,000. Iron meteorites tend to have Ga and Ge concentrations within various restricted ranges—thus their usefulness for the classification of iron meteorites. No other element correlates strongly with Ga and Ge. Here we point out parallels between the abundance patterns of moderately volatile elements in iron meteorites and ordinary chondrites, and discuss how these can be related to condensation processes in the solar nebula.

Most (83%) of the irons have Ge/Ni ratios in the range $(0.2-6.3) \times 10^{-3}$ atoms/atom, in the same range as ratios in primitive, volatile-rich CI chondrites, 2.6×10^{-3} atoms/atom and in other groups of chondritic meteorites^{1,2}. Two iron meteorite groups show much lower Ge/Ni ratios: $(1.0-1.2) \times 10^{-6}$ atoms/atom in Group IVA and $(1.6-2.9) \times 10^{-7}$ in Group IVB. The processes responsible for these very low Ge concentrations were clearly much more effective than those that affected the chondritic meteorites.

Concentrations of Ga and Ge in iron meteorites were probably established either during condensation³ at the nebular location where that parent body formed and/or during processes occurring within each parent body.

Wai *et al.*^{4,5} showed that core formation in a planetary body does not result in fractionation of Ge from Ni. By default, it seems that the fractionation of Ge and Ga from Ni must have occurred during the condensation and agglomeration of the solar nebula.

Gallium and germanium belong to a group of moderately volatile elements having abundances 1.2-10 times lower in ordinary chondrites than in CI chondrites, which are generally believed to be most representative of mean Solar System abundances⁶. It is instructive to compare Ga and Ge with two other moderately volatile elements, As and Cu. In Fig. 1 data for Group IVA and IVB irons are compared with IIIAB irons and H-group ordinary chondrites; element/Ni ratios are normalised to CI chondrites.

The H/CI ratios in Fig. 1 show a steady decrease from As to Ge; Wasson and Chou⁶ suggested that nebular condensation temperatures decrease similarly, and that a nebular mechanism existed which resulted in increasing loss of gas with decreasing condensation temperature. Wasson and Chou discussed several nebular processes which could result in such loss patterns.

Figure 2 shows equilibrium condensation activities of the most stable compounds of Cu, Ga and Ge at nebular pressures of 10^{-4} and 10^{-6} atm. No calculations are reported for As, since data could not be found for a number of potentially important compounds. Equilibrium between gases and solids and ideal solution was assumed. Mean Solar System abundances of C, H and O were taken from Cameron⁷, and for Cu, Ga and Ge from Wasson and Chou⁶. Free energy data were taken from the JANAF (1971) tables for CO, H₂O and Cu compounds⁸, and from other recent sources for Ga (refs 9 and 10) and Ge (refs 11-14).

In a departure from earlier calculations regarding trace element condensation^{15,16}, the ordinate does not show the fraction of the element removed from the gas phase, but rather the equilibrium activity in each phase divided by that expected if the element condensed entirely in this phase. This representation allows more insight into the siting of each element during equilibrium processes in the nebula. For the six phases of importance these normalising CI ratios were: Cu metal, Cu/(Fe+Ni) = 6.1×10^{-4} ; CuS_{0.5},

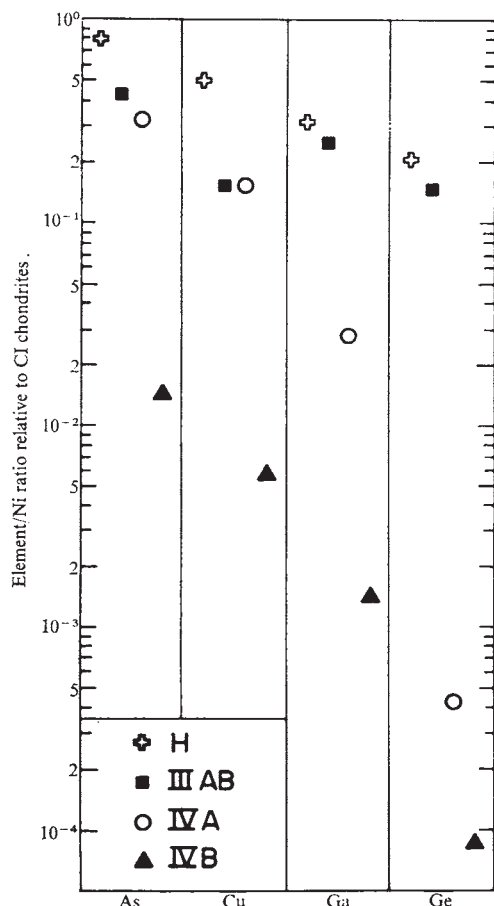


Fig. 1 CI chondrite normalised element/Ni ratios of four moderately volatile elements are generally similar in H-group chondrites and IIIAB irons. The IVB irons have very low ratios of each volatile, whereas the IVA irons have normal amounts of two volatiles (As and Cu) and very low amounts of two others (Ga and Ge).

unity above 680 K, $\text{Cu/S} = 5.4 \times 10^{-3}$ below 680 K; Ga metal, $\text{Ga}/(\text{Fe} + \text{Ni}) = 4.3 \times 10^{-5}$; $\text{GaO}_{1.5}$, $\text{Ga/Al} = 4.8 \times 10^{-4}$; Ge metal, $\text{Ge}/(\text{Fe} + \text{Ni}) = 1.3 \times 10^{-4}$; Mg_2GeO_4 , $\text{Ge/Si} = 1.2 \times 10^{-4}$. An implicit assumption of this treatment is that unit activity of a trace element results from complete substitution for the element in the denominator (the exception is Cu for Fe in FeS).

Scott¹⁷ noted that the IVB irons are enriched in refractories (Re, Os, Ir) and depleted in volatiles and concluded that they represent high temperature nebular condensates. The data in Fig. 1 cannot be explained by equilibrium condensation processes, however. This conclusion is independent of pressure, but can be conveniently demonstrated by consideration of the condensation curves at a pressure of 10^{-4} atm (Fig. 2a). In order that only 0.6% Cu condenses, equilibration with nebular gases must cease at a temperature of about 1,350 K. At this temperature, however, the equilibrium Ga and Ge activity ratios are 2.2×10^{-4} and 5.3×10^{-5} , substantially lower than IVB values. Kelly and Larimer³ reached a similar conclusion.

The moderately volatile element pattern in IVB is in fact quite similar to that in ordinary chondrites (Fig. 1). The difference is one of degree, implying that the same loss mechanisms were responsible but that the IVB process was much more efficient.

A plausible picture is the following. Condensation at the Group IVB location occurred during a period when the temperature decreased rapidly. Diffusion was too slow to allow trace metals to reach the centres of Fe-Ni grains, and solution occurred only in the outermost atomic layers.

As temperatures continued to fall, trace metals condensed as a fine aerosol that was later blown away by a T-Tauri solar storm. The IVB irons have high Ni contents suggesting that an appreciable amount of Ni-poor metal was lost, possibly by the same mechanism. Condensation at the H-group location was more complete because the temperature decreased less rapidly, and trace metals could diffuse deeper into Fe-Ni grains.

As noted by Scott¹⁷ the abundances of As and Cu in Group IVA irons are nearly identical to those in Group IIIAB, whereas Ga and Ge are lower in IVA by factors of about 10 and 300, respectively. The IIIAB abundances are nearly identical to those in the H group, and it is likely that the same process was responsible for the loss of moderately volatile elements at both nebula locations.

At the IVA location conditions were also similar as the nebula cooled to the condensation temperature of Cu, but below this temperature condensation was much less complete. We can think of two ways in which IVA conditions may have differed from IIIAB or H conditions: either (1) the rate of temperature decrease increased abruptly below the Cu condensation temperature, and incomplete condensation and loss of Ga and Ge occurred in conditions similar to those proposed for the loss of volatiles at the IVB location; or (2) early condensation occurred in equilibrium conditions, but below some limiting temperature the gas and solids became physically separated and no further condensation was possible³. If the second mechanism is viable, we should find temperatures in Fig. 2 which account for the Ga and Ge depletions in IVA relative to those in Group IIIAB. At a pressure of 10^{-4} atm 1,060 K could provide the correct factors. At 10^{-6} atm no temperature exactly matches the IVA/IIIAB depletions; the best fits are obtained at about 820 and 900 K. This double value results from the tendency of Ga preferentially to enter the silicates below 860 K. The temperature of equilibration with nebular gases cannot have been less than 820 K, since lower temperatures result in sharp increases in the Ge/Ga ratio in Fe-Ni. This lower temperature is implausible, however, since the Ga concentration in the silicates is high, and this Ga should re-enter the metal at the high temperatures needed to bring about gravitational separation of metal from silicate in order to form a core.

Determination of a siderophilic element (that is, one tending to remain in the metallic state) more volatile than Ge offers a means for choosing among the two IVA models. The model involving a change in cooling rate should result in higher concentrations of such an element than that incorporating a minimum equilibration temperature. Unfortunately, the same atomic forces that cause some elements to show siderophilic properties tend to produce moderately high nebular condensation temperatures. We know of no siderophilic element more volatile than Ge. The most siderophilic of the more volatile elements is Zn, which has a condensation temperature close to 680 K. Published data on Zn show considerable scatter, but it seems that metal/sulphide concentration ratios can be as high as 0.1. Smales *et al.*¹⁸ reported Zn concentrations less than their detection limit of $1 \mu\text{g g}^{-1}$ in IVA irons. We are initiating a neutron activation study of Zn in these meteorites which will allow detection down to ng g^{-1} levels.

Although S has a pressure-independent condensation temperature (680 K, Fig. 2) lower than that of Ge at 10^{-4} atm, and the same as Ge at 10^{-6} atm, CI normalised S/Ni ratios in IVA and IVB irons are $\sim 1 \times 10^{-2}$ and 7×10^{-4} , respectively—factors of 8 and 20 higher than those of Ge. This difference may result in part from the differences in condensation reactions. Whereas Ge and Ga can condense as pure metals, S must form FeS by reaction with the surfaces of metal grains. The latter reaction should proceed rapidly until all grains are coated with

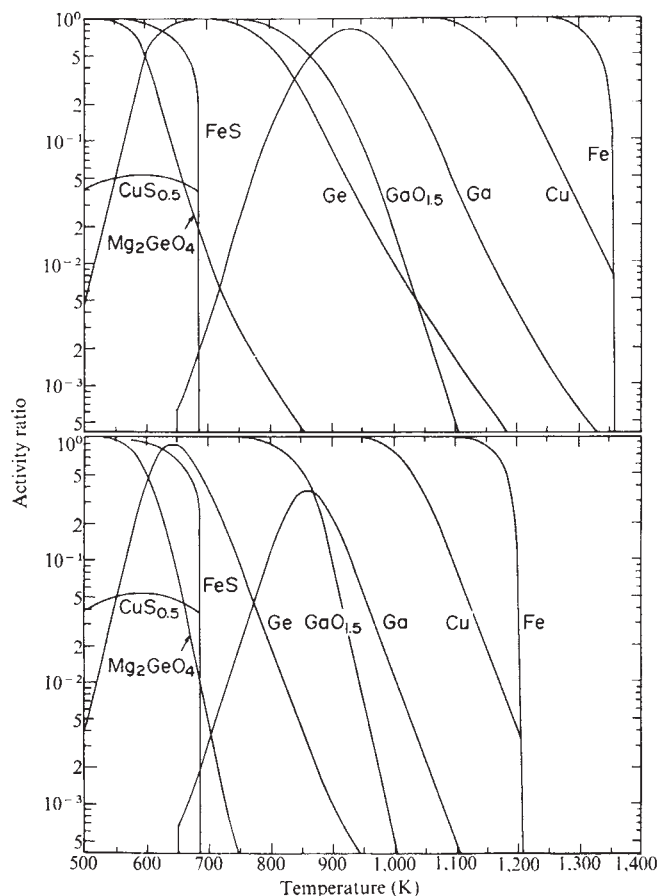


Fig. 2 Nebular condensation of Cu, Ga and Ge at pressures of 10^{-4} atm (a) and 10^{-6} atm (b); the ordinate gives the equilibrium activity of the substance divided by that expected from full condensation of the trace element in this chemical form. At high temperatures Ga and Ge condense as the metal but at low temperatures the oxide form becomes dominant. Some Cu enters FeS when the latter becomes a stable phase at 685 K.

FeS. At moderate temperatures (>500 K) there is no other reaction by which an appreciable fraction of the S can condense, thus it cannot undergo homogeneous condensation as a fine aerosol capable of being blown away by a T-Tauri wind.

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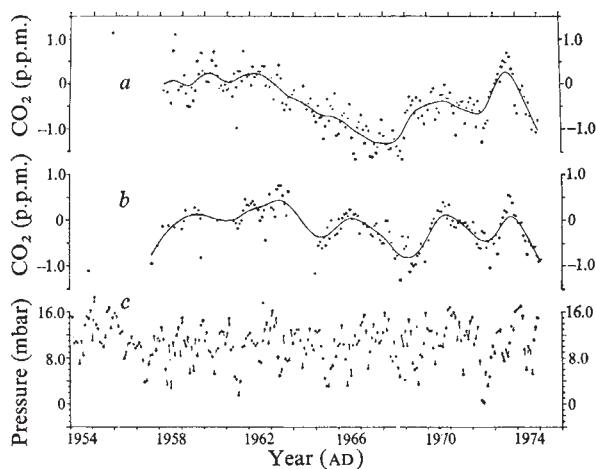
Modulation of atmospheric carbon dioxide by the Southern Oscillation

ATMOSPHERIC CO_2 records for the South Pole and Mauna Loa Observatory, Hawaii, show a seasonal variation, presumably arising from the uptake and release of CO_2 by vegetation, and a long term increase, almost certainly caused by combustion of fossil fuel. The increase is much greater in some years than in others^{1,2}. Changes in the rate of fossil fuel combustion are not likely to be the cause of the variation in yearly increase, as combustion has increased very steadily³. I present here evidence that the variation is connected to the Southern Oscillation, a large scale atmospheric and hydrospheric fluctuation with an irregular period of 1–5 yr (ref. 4). The connection, if present, indicates that a principal cause of the variation may be a change in the rate of removal of CO_2 by the oceans.

The CO_2 records, approximately as they would appear if there were no seasonal effect and no long term increase, are shown in Fig. 1. These 'anomaly curves' were obtained by an iterative procedure in which the data were first fitted to a long term increase and a seasonal effect, represented, respectively, by a spline function⁵ (with stiffness set by eye) and by an average over identical months, for each year, of the difference between the data and the spline. A constant airborne fraction multiplied by an integral of the fossil fuel consumption rate was then subtracted from the spline (long term increase) to remove the effect of fossil fuel combustion. The analysis will be discussed in more detail elsewhere.

The difference in average monthly barometric pressures between Easter Island and Darwin, Australia, is a suitable indicator⁶ of the Southern Oscillation, and will be called the Southern Oscillation Index (SOI). Easter Island is near the centre of a subtropical high pressure cell, and Darwin is representative of the equatorial low pressure zone. The SOI is directly related to the strength of the easterly trade winds that blow along the Equator. When the SOI is high, the trade winds are stronger than average. The middle latitude westerlies along the southern flank of the subtropical

Fig. 1 Atmospheric CO_2 records at a, Mauna Loa, Hawaii and b, South Pole, with seasonal effect and fossil fuel increase removed by a procedure described in the text. c, Southern Oscillation Index. Data assembled by W. H. Quinn, Oregon State University.



anticyclone belt also tend to blow more strongly. The Humboldt Current and equatorial currents are accelerated, thereby increasing upwelling along the coast of South America and along the Equator. The equatorial counter-current, which flows against the trade winds, is decelerated. Low SOI brings reduced trade winds and reduced middle-latitude westerlies. The equatorial countercurrent flows more strongly and carries warmer water to the eastern Pacific⁷. Low SOI is often accompanied by El Niño, an invasion of warm water off the west coast of northern South America, which brings disaster to the fishing industry and torrential rain to a normally arid region.

Correlation maxima⁸ for the South Pole anomaly with the smoothed SOI (12-month moving average) near zero lag are 0.40 with a positive SOI lag of 3 months and -0.45 with a negative SOI lag of 17 months (Fig. 2). If these two time series were uncorrelated, the cross-correlation function would be expected to be zero, with a standard deviation, near zero lag, of approximately 0.29. The statistical confidence level for the existence of a connection is thus about 83%. The correlation maxima with positive SOI lag of three months would not seem to meet the requirement of a causal relationship, since one would expect the Southern Oscillation to be the cause of change in atmospheric CO₂ level, and not the reverse. (The possibility exists that the anomaly in CO₂ level drives the Southern Oscillation, but it seems unlikely because the CO₂ changes are only 1 part per million (p.p.m.) in a total of about 330 p.p.m.) The negative, 17-month SOI lag is physically possible, but rather long. Corresponding correlation maxima for the Mauna Loa anomaly are smaller: 0.14 with an SOI lag of 6 months, and -0.28 with a negative SOI lag of 10 months. The standard deviation, if the series were unconnected, would be 0.18 near zero lag. Consequently, none of these correlation maxima is entirely satisfactory.

An atmosphere and hydrospheric circulation phenomenon should directly affect the rate of change of the CO₂ level instead of the level itself. For example, the transfer of CO₂ across the air-sea interface is governed approximately by an equation of the form

$$-(dp/dt) = k(p-P) \quad (1)$$

where p is the CO₂ partial pressure in the atmosphere, P is the CO₂ partial pressure in the ocean beneath a thin boundary layer, and k is a rate factor which depends strongly on the boundary layer thickness, and consequently on wind shear⁹. It is therefore reasonable to correlate the derivatives of the anomaly curves with the smoothed SOI (Fig. 3).

Correlation maxima are then -0.64 with a negative SOI lag of 6 months for the South Pole, and -0.55 with a negative SOI lag of 2.5 months for Mauna Loa. Standard deviations near zero lag would be 0.34 and 0.28, respectively, if the series were uncorrelated. Not only are the derivative anomaly correlations higher, relative to the standard deviations, but the time lags are more reasonable.

The nominal confidence level for the existence of a connection between the time derivatives of the anomalies and the Southern Oscillation is about 95%. The true confidence level, however, is probably smaller because of the extensive data manipulation and smoothing. Longer CO₂ records should resolve whether a connection exists.

The influence of the Southern Oscillation on the atmospheric CO₂ level seems to be felt 3.5 months earlier at Mauna Loa than at the South Pole. The reason for this may be that the South Pole is in a relatively fixed high pressure zone, isolated from the lower latitudes by circumpolar winds¹⁰.

Increased loss of CO₂ from the atmosphere during periods of high SOI must be attributable to increased uptake by one of the two natural reservoirs with which atmospheric CO₂ principally exchanges: the oceans and the biota. If a high

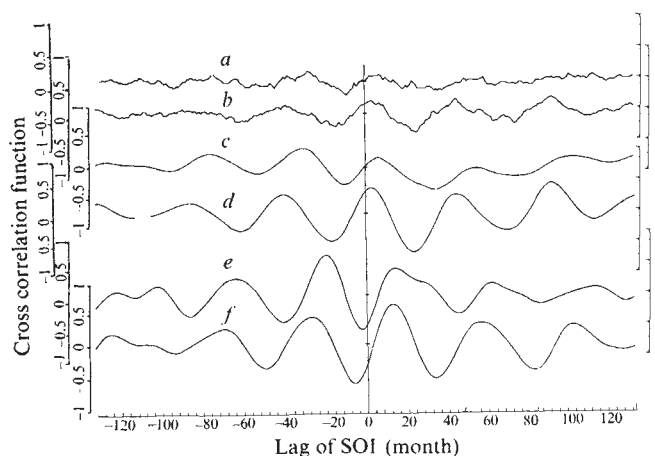


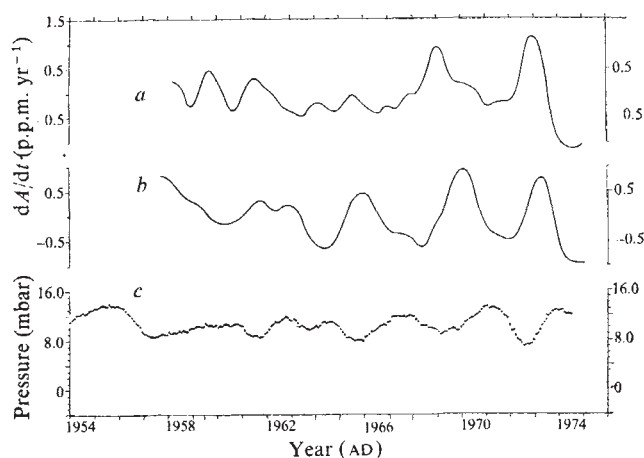
Fig. 2 Cross correlation functions for Mauna Loa and South Pole CO₂ records and the SOI: *a*, unsmoothed Mauna Loa anomaly data (with missing data filled in by interpolation) and unsmoothed SOI; *b*, unsmoothed South Pole anomaly data (with missing data filled in by interpolation), and unsmoothed SOI; *c*, Mauna Loa anomaly curve and smoothed (12-month moving average) SOI; *d*, South Pole anomaly curve and smoothed SOI; *e*, derivative Mauna Loa anomaly curve and smoothed SOI; *f*, derivative South Pole anomaly curve and smoothed SOI. All correlation functions are plotted against the lag of the SOI. Standard deviations, if each pair of curves were uncorrelated, would be (*a-f*): 0.09, 0.12, 0.18, 0.29, 0.28, and 0.34.

SOI were associated with increased rainfall over arid but otherwise fertile land, increased vegetative uptake could explain the correlations. Data to test this hypothesis are probably available, but have not been examined in the course of this study. The large areas of land that would have to be involved make this explanation seem unlikely, however.

The oceans transfer CO₂ to the atmosphere in regions where there is upwelling: along the Equator and along the western margins of continents, and absorb CO₂ from the atmosphere in many other regions where the CO₂ partial pressure exerted by the oceans is less than that of the atmosphere¹¹.

The Southern Oscillation has global influence¹², and indeed may be a fluctuation in the intensity of the general atmospheric circulation. Kanwisher has shown that the CO₂ exchange rate (essentially the coefficient k in equation (1)) varies as the square of the wind speed⁹, so a small increase

Fig. 3 Time derivative CO₂ records at *a*, Mauna Loa and *b*, South Pole, obtained by differentiating corresponding curves in Fig. 1. *c*, SOI, smoothed by a 12-month moving average, shown for comparison.



in average wind speed at high SOI could cause increased uptake by the oceans.

If the Southern Oscillation is mainly localised in the South Pacific, the explanation may be that increased ocean uptake occurs in the zone of westerly winds from ~ 40 – 50° S. This region should have a very high exchange rate for CO_2 because of the strong winds and large ocean area⁹, and the westerly winds in this zone correlate with the easterly trade winds to the north¹⁰, and thus with the SOI.

On the other hand, a high SOI should also bring increased upwelling, at least along the western coast of South America and along the equatorial Pacific. The observed correlations seem to indicate that the stronger absorption by the oceans dominates the effect of the increased upwelling.

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D-region rocket measurements in winter anomaly absorption conditions

THE winter anomaly in ionospheric absorption was first identified by Appleton¹, and although extensively studied over the past 40 yr it still remains for the most part a major unsolved problem. Its principal feature is that at latitudes above $\sim 35^\circ$, on most winter days, h.f. ionospheric absorption is large (often larger than on summer days at 1200). Furthermore, there is considerably greater variability in the day-to-day absorption in winter than in summer, and the magnitude of the anomaly seems to increase with latitude, although at high latitudes the phenomenon is largely masked by other disturbances characteristic of the auroral zone. Rocket measurements have shown that on winter anomaly days there is a considerable enhancement of the electron density of the lower ionosphere particularly in the height range 80–90 km (refs 2–4). Calculations indicate that these enhanced electron densities would be sufficient to account for the winter anomaly absorption without involving any changes in other parameters which determine the magnitude of h.f. absorption such as the collisional frequency ν .

On a recent coordinated firing of three Petrel payloads from South Uist (57.4° N, 7.4° W) at approximately 1200 on November 29, 1974 we measured ion and electron concentrations on a day of very marked winter anomaly absorption. The first rocket was instrumented by the Max-Planck-Institut, Heidelberg and carried a quadrupole mass spectrometer to measure positive ions and neutral constituents. The second rocket was instrumented by the Appleton Laboratory to measure atomic oxygen and also carried a Langmuir probe to measure relative values of the electron concentration. The third rocket was instrumented by the

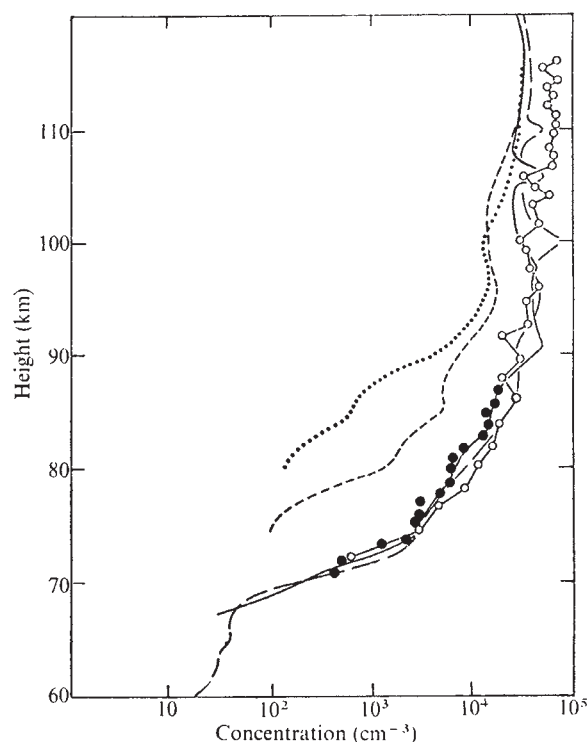


Fig. 1 Comparison of NO^+ ion and electron concentrations (Ne) during severe winter anomaly absorption at South Uist, Scotland (57.20° N, 7.14° W). Also shown are electron concentrations for normal winter and summer conditions at similar solar zenith angles χ . November 29, 1974—winter anomaly day, $\chi \approx 80^\circ$: $\circ-\circ-\circ$, NO^+ flight, P182H, 1117 UT; $-\cdot-\cdot-$, Ne (probe), P175H, 1153 UT; $-\cdot-\cdot-$, Ne (probe), P179H, 1309 UT; $\bullet-\bullet-\bullet$, Ne (Faraday rotation) P179H. December 6, 1971, normal winter day, $\chi \approx 83^\circ$: $-\cdot-\cdot-$, Ne (Faraday rotation + probe), P83H, 1402 UT. June 26, 1971, summer day, $\chi \approx 80^\circ$: $\cdots$, Ne (Faraday rotation + probe), P120H, 1944 UT.

University College of Wales, Aberystwyth and carried a Faraday rotation radio experiment on two frequencies (which provided absolute measurements of the electron concentration) and also carried a Langmuir probe identical to that flown on the second rocket. Mass spectrometer measurements of positive ion composition were obtained over a height range of 70–115 km and it was observed that above 72 km NO^+ was by far the most abundant species. This is in contrast to the normal situation in which O_2^+ ions reach abundances comparable with NO^+ above about 85 km, and water cluster ions are dominant below that height². Relative measures of electron density by the Langmuir probes were obtained from 60 to 130 km and absolute values by the Faraday rotation technique were obtained from 70 to 90 km. These various results are shown in Fig. 1. For comparison purposes Fig. 1 also shows electron density curves for about the same solar zenith angle but measured (1) on a winter day of below average absorption and (2) on a summer day.

It can be seen that throughout the height range 70–115 km the profile of enhanced electron density on the winter anomaly day is practically coincident with the profile of NO^+ concentration, a result which points strongly to the conclusion that winter anomaly absorption occurs as a result of a greatly enhanced concentration of NO^+ particularly at D-region heights.

Earlier work on the spatial and temporal morphology of the winter anomaly absorption phenomenon has indicated that its incidence may well be associated with the occurrence of some fairly large scale atmospheric movements in the lower ionosphere. If further experimental results support the conclusion indicated by the present rocket results

concerning the role of NO, and if further progress with the problem is to be made, an explanation will be needed for the enhanced production of NO and/or its transport to the locations concerned during the winter months.

A fuller account of this work and of other results obtained in these rocket flights will be published elsewhere.

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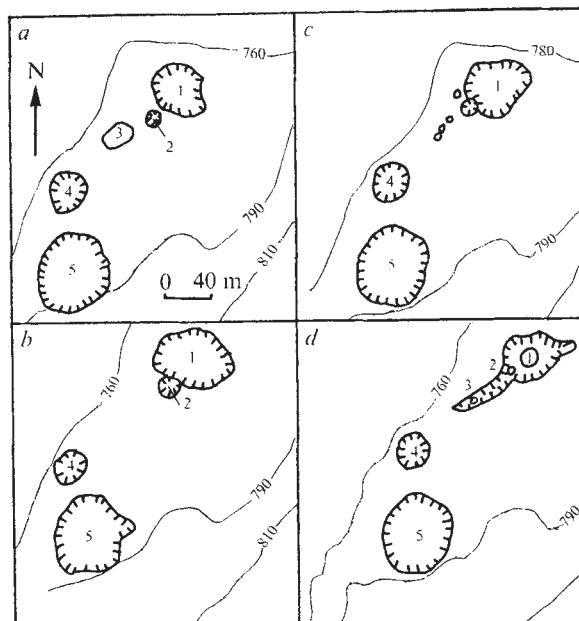


Fig. 2 Evolution of the crater plain (Fossa): a, October 1972; b, May 1973; c, May 1974; d, December 1974. Since the explosions of September 1974 vents 1, 2, 3 are all inside an elongated crater (d).

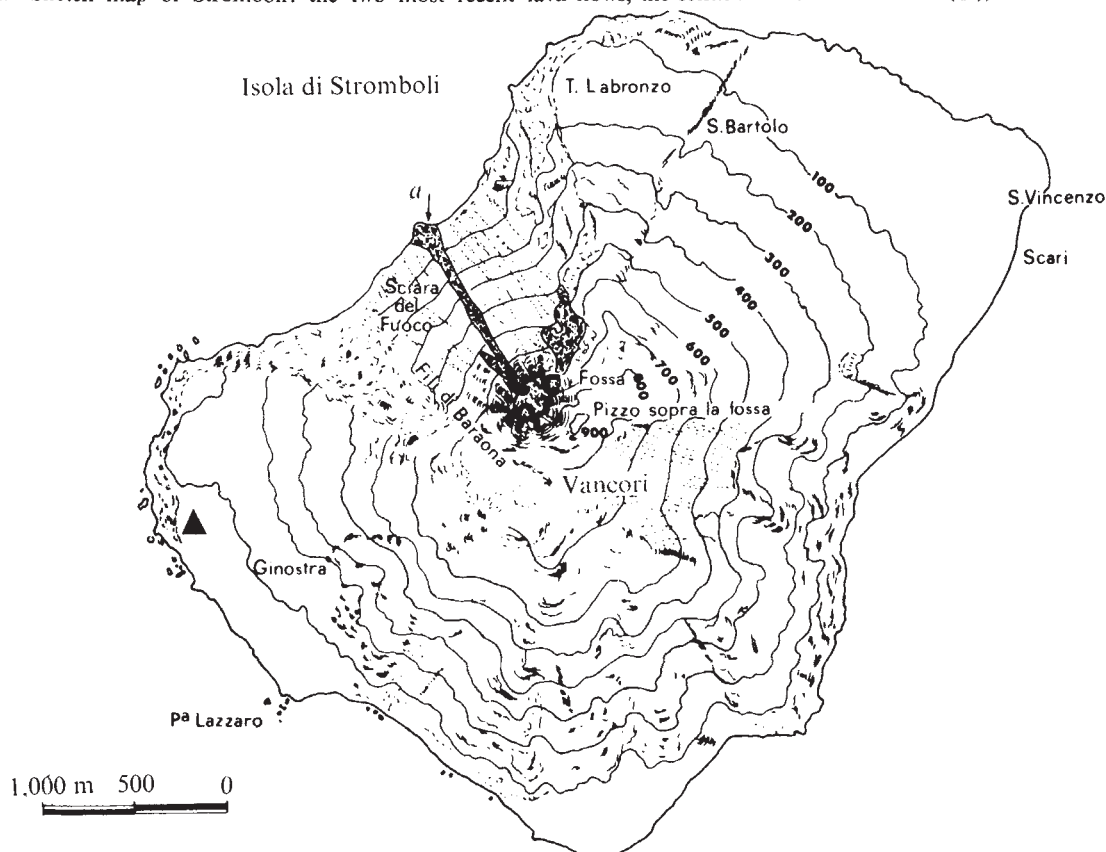
Recent activity of Stromboli

DURING a recent extensive phase¹ on Stromboli (November 5–24, 1975; Fig. 1), which was accompanied by an increase of explosive activity, I noticed an interesting inverse relationship between seismic and eruptive activity.

The recent extensive phase was the first since 1971. In October 1972 the crater terrace was an oval shaped basin with five vents, aligned along a NE–SW fissure (Fig. 2a); four of the vents were active. In December 1972 two strong

explosions occurred, and large fragments of old lava fell near San Bartolo. In April 1974 a fissure appeared in the central part of the 'Fossa'; three cones were built by the explosive activity along this fissure between May and August (Fig. 2c). On September 19, 1974 at 0010 GMT two big explosions occurred; after the explosions I observed that the cinder cones had been destroyed and that an irregular elongated crater with three vents had taken their place (Fig. 2d).

Fig. 1 Sketch map of Stromboli: the two most recent lava flows, the seismic station of Ginostra (▲), and 1975 lava flow.



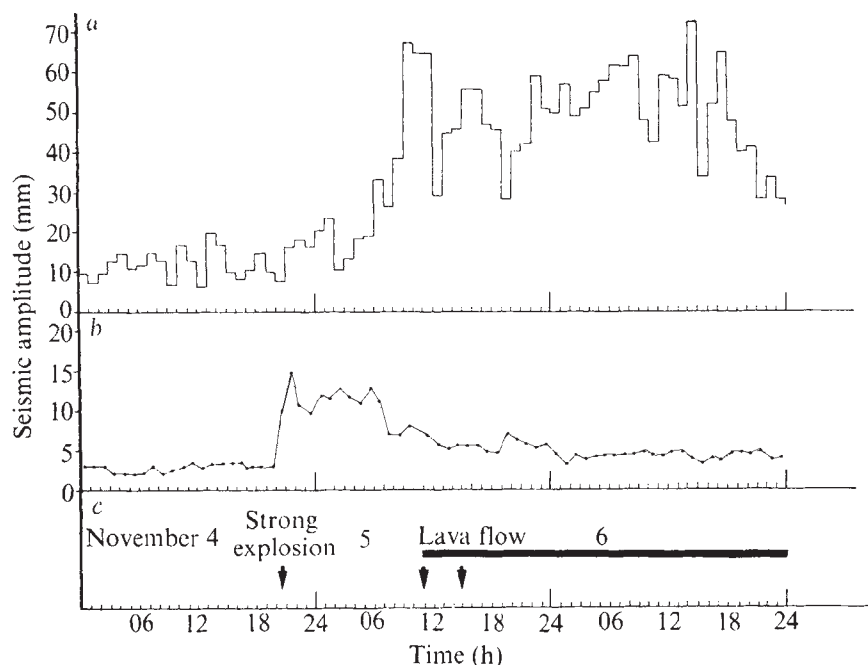


Fig. 3 a, Hourly frequencies of seismic events; b, relative amplitude of tremors; c, eruptive activity.

The earliest lavas produced were observed on November 5, 1975 at 1500. At that time the two flow fronts were at 400 m OD and 500 m OD on the Sciara del Fuoco, respectively. The first flow originated from a new bocca at 700 m OD on the Sciara del Fuoco; the second flowed from a collapse of the crater rim originating from a vent within the main crater close to the lip (Fig. 1). This flow stopped on November 6. The flow from the bocca on the Sciara del Fuoco reached the sea on November 5 at 1800.

The lava flow down the Sciara was 10 m wide, but at the Sciara base where the lava flowed into the sea, the flow formed a delta-like body with a front about 100 m wide. Only a small part of this material was molten lava, flanked with fragments of lava pushed aside during flow along the Sciara. The effusive activity stopped on November 24.

The explosive activity reached its peak between November 5 and November 7 (when more than one explosion occurred every second). Two of the vents inside the elongated crater (Crater 1, Fig. 2d) were very active. Also active were vents 4 and 5. Crater 1 was characterised by fire fountaining and explosions. The explosions threw lava fragments and scoriae to a maximum height of 700 m and there was rumbling within the crater. Vent 5 was also characterised by explosions with maximum projectile heights of about 400 m. The explosions from Vent 4 ejected ash to about 800 m above the crater lip. During this period of activity 65% of the explosions were from Crater 1 and 21% and 12%, respectively, from Vents 4 and 5. On November 10 and 11 the ejection of ash had replaced the ejection of molten fragmenta and scoriae. Only two craters were active, Crater 1, in which 52% of the explosions occurred, and Crater 5, in which 22% of the explosions occurred; the other 26% was represented by weak explosions (or very shallow earthquakes?) recorded only on the seismograph at Ginostra. On November 13 only the ejection of ash occurred from Craters 1 and 5.

The seismic activity of Stromboli Volcano is recorded at a station at Ginostra, located about 2 km west of the crater plain. The station includes a 1-Hz vertical geophone, and the amplified signal is sent by telemetry to the Osservatorio Geofisico di Lipari. There, the signal is recorded on magnetic tape and monitored. Generally, the seismic activity of Stromboli comprises single seismic events superimposed on a continuous microtremor. On the evening of November 4, 1975, the amplitude of volcanic tremor in-

creased sharply from 2000, and suddenly decreased at 0700 on November 5 (Fig. 3b). At that time the hourly frequencies of the seismic events increased (Fig. 3a). Figure 3 shows that the increase of the volcanic tremor began 11 h before the beginning of eruptive paroxysm. On the morning of November 7, in order to determine the nature of the single events recorded, observations were conducted for 1 h at the crater plain. Around 99% of the events recorded were related to observed explosions. During later observations (November 10, 11, and 13) the proportions of seismic events related to explosions were 65%, 42% and 35%, respectively. This change must be related to the fact that the level of the magmatic column became progressively lower. In fact, after November 10 the products of explosion appeared on the crater lip more than 10 s after the seismic event was recorded.

The seismic record during this recent activity of Stromboli confirms that the source of the seismic energy is located within the volcanic superstructure, that an inverse relationship between the amplitude of microtremors and number of events per unit time exists (Fig. 3), and that the seismic events can be clearly related to events in the crater. Such a clear relationship between events in the crater, and seismic activity recorded, suggests that major changes of crater activity can be detected by the existing seismic network.

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² Lo Bascio, A., Luongo, G., and Nappi, G., *Bull. volcan.*, 37, 597-606 (1973).

³ Nappi, G., *Boll. Soc. geol. ital.*, 94 (in the press).

Sulphate-light scattering ratio as an index of the role of sulphur in tropospheric optics

THE optical properties of the non-cloudy atmosphere are determined by a fixed component, the molecular gases, and a variable component, the aerosol particles. The optical effects of these two components have been subject to speculation and

scientific investigation for many years. The major features of molecular and particle optics are reasonably well understood¹⁻³ and instrumental techniques have been developed to measure extinction of light⁴, scattering phase function^{5,6} and the scattering component of extinction⁷. Few data or analyses have, however, been presented which consider the chemical nature of the aerosol in terms of its effects on radiative properties, although a few exceptions can be cited⁸⁻¹². Experimental evidence quantitatively linking the chemical composition of aerosol particles with the macroscopic optical consequences is sparse, but here we mention one approach relating chemical and optical measurements on regional scale air masses and present preliminary data.

Measurements^{13,14} have shown that the aerosol particle volume distribution is usually bimodal, and that the two modes are approximately log normal, are of mass mean diameters 0.4–0.8 μm and 8–20 μm and geometric standard deviations of about 2. Mie calculations show that the small particle mode usually dominates the optical scattering. Further, the measured particle volume concentration in the range 0.1–1.0 μm is often highly correlated to the extinction due to scattering by particles as measured with an integrating nephelometer with a correlation coefficient as high as 0.95 (ref. 14). Chemical analyses have shown that the small particle mode is often dominated by few substances notably non-maritime sulphates, nitrates, ammonium and condensed organic material^{8,15,16}. Measurements in and near St Louis, Missouri¹⁷ showed that sulphates often accounted for $\geq 50\%$ of the mass of material in the diameter range $0.1 \geq D_p \geq 1 \mu\text{m}$.

Dry particle light scattering and the possibly dominant chemical quantity (sulphate compounds) seem the most useful parameters to measure. These measurements may be compared with other atmospheric data, with laboratory values for simulated systems and with Mie theory to provide insight into the fraction of optical effect attributable to sulphate components. The key ratio is that of sulphate concentration to light scattering extinction coefficient due to dry particles, $[\text{SO}_4^{2-}]/b_{sp}$, g m^{-2} .

Table 1 provides a summary of various schemes which can be used for this evaluation of the mass concentration/ b_{sp} and/or $[\text{SO}_4^{2-}]/b_{sp}$ ratio. It should be noted, of course, that "sulphate" is not a compound itself and must have a cation. The lightest cation is H^+ and it is found in atmospheric particles some of the

time. One of the heavier ions is NH_4^+ as in $(\text{NH}_4)_2\text{SO}_4$, which is also frequently found as an optically dominant component (that is, 50 mol %)¹⁷. Since the ratio of molecular weight of $(\text{NH}_4)_2\text{SO}_4$ to that of SO_4^{2-} is only about 1.5, and that of H_2SO_4 is almost 1, the use of SO_4^{2-} mass concentration alone for an index is warranted at the outset. This simplification, which is due, in many cases, to a lack of knowledge, of the cation associated with sulphate, must be kept in mind as it produces an inherent uncertainty of about $\pm 25\%$ in the ratio.

The data pairs for the ratio were obtained from aircraft flights, mainly over southern Sweden. An integrating nephelometer²³ was used to measure the extinction coefficient due to dry particles at a given wavelength (in most cases 450 nm) and a filter was used to obtain samples for postanalysis of the SO_4^{2-} content by the thorin method. Incoming air was heated to $\sim 20^\circ\text{C}$, providing relative humidities below 50%. Some flight paths and altitudes were selected beforehand to provide air from populated areas of continental Europe as part of the study by the Organization for Economic Cooperation and Development of sulphur transport across national boundaries *vis-à-vis* the problem of acidic rain (Long Range Transport of Air Pollutants project), whereas others were deliberately made at random. Most flights were at altitudes above 300–1,000 m and well inland (250 km) so that large particle ($D_p > 1 \mu\text{m}$) maritime sulphate concentrations should be small or non-existent. The average sulphate concentrations ranged up to $35 \mu\text{g m}^{-3}$, significantly higher than the maritime SO_4^{2-} in this area (typically less than 3–10 $\mu\text{g m}^{-3}$ based on Cl^- analysis) and provided an adequate dynamic range for correlation calculations.

Table 2 includes all ratios of the $[\text{SO}_4^{2-}]$ and b_{sp} , and summarizes the correlation calculations.

(1) The $[\text{SO}_4^{2-}]/b_{sp}$ ratio from the overall regression is 0.2 g m^{-2} which is comparable with the measured and predicted ratios in Table 1 for the ratio of submicrometre mass concentration to b_{sp} .

(2) The correlation between $[\text{SO}_4^{2-}]$ and b_{sp} for all data is 0.73, indicating that a large fraction of the variance of b_{sp} can be associated with $[\text{SO}_4^{2-}]$.

(3) The correlation between $[\text{SO}_4^{2-}]$ and b_{sp} for individual days (that is, flights) ranges from $r=0.99$ to $r=0.24$. Five of nine cases with three or more data points had $r > 0.86$, whereas the rest had $r < 0.60$. Trajectory calculations showed that the

Table 1 Ratio of volume or mass concentration of submicrometre particles to light scattering

Basis	Modal volume ratio, v/b_{sp} ($\text{cm}^3 \text{ m}^{-2}$)	Modal mass ratio, m/b_{sp} (g m^{-2})	Volume or mass correlation to b_{sp}	Reference
(1) Measured volume concentration in diameter range $0.1 \geq D_p \geq 1.0 \mu\text{m}$ and measured light scattering coefficient (b_{sp}), from several suburban locations in southern California	0.1	0.2*	0.95	14
(2) Mie calculations using a log normal size distribution of geometric mean diameter by volume = 0.6 μm , geometric s.d. = 2, refractive index = 1.5	0.15	0.3*	—	18
(3) Measured volume concentration with $0.1 \geq D_p \geq 1.0 \mu\text{m}$ and measured b_{sp} , from Pasadena, California	0.08	0.16*	0.82	19
(4) Measured volume concentration with $D_p < 2 \mu\text{m}$ in urban-influenced (urban district) and aged polluted cases	0.05–0.15	0.10–0.3*	—	14
(5) From direct measurement of total mass concentration and b_{sp} , estimation that half of mass is in optically active range $0.1 \leq D_p \leq 1.0 \mu\text{m}$	—	0.2	0.82	20
(6) From direct measurement of total submicrometre mass concentration and b_{sp} , from St Louis, Missouri	—	0.22	—	15, 17
(7) $[\text{SO}_4^{2-}]/b_{sp}$, from St Louis, Missouri	—	0.1	—	15, 17
(8) Calculated extinction coefficient for a model aerosol of $(\text{NH}_4)_2\text{SO}_4$ at 70% r.h.	—	0.1 up to 0.2	—	21
(9) Laboratory aerosol of $(\text{NH}_4)_2\text{SO}_4$ generated by sparger $r_{gm} = 0.6 \mu\text{m}$, $\sigma_g = 1.43$	—	0.097+0.007	—	22

*Particle density assumed to be 2 g cm^{-3}

Table 2 Measured ratios and correlations

Year	Month	Day	Local time	N	r	[SO ₄ ²⁻]/b _{sp} § (g m ⁻²)	Trajectory
1973	May	9	1300-1515	7 (6)*	0.54 (0.96)*	0.21	
	May	23	1245-1500	7	0.99	0.11	
	June	13	1400-1600	5	0.97	0.15	
	June	28	1345-1430	2			
	July	4	1420-1650	4 (3)*	0.60 (0.93)*	0.16*	
	December	20	1500-2100	9	0.93	0.38	
1974	January	3	1214-1650	3	0.99	0.49	
	March	14	1210-1410	8	0.50	0.07	
	March	19	1025-1100	2		0.10	
	March	21	1340-1430	2		0.45	
	May	3	1045-1245	6	0.48	0.15	¶
	May	16	1115-1330	8	0.35	0.17	¶
	June	5	1030-1230	10	0.24†	0.06	¶
	May	28	1227-1437	4	0.86‡	0.08	
	Total			77	0.73	0.2	

N, Number of samples.

r, Linear correlation coefficient.

*If one data point is omitted.

†SO₄²⁻ concentration is too low to permit accurate readings.

‡Measurements at 530 nm.

§Least square estimate (calculated as $k = \Sigma ([SO_4^{2-}]/b_{sp})/\Sigma (b_{sp})^2$).

||Trajectory from south-east, south, south-west, west.

¶Trajectory from north-west, north, north-east, east.

high correlation cases occurred with flow from the south-east, south and south-west whereas the low correlation cases were from the north-west, north and north-east.

(4) The [SO₄²⁻]/b_{sp} ratio is comparable with that found in St Louis, Missouri.

(5) If the size distribution were known, more definite conclusions could be made regarding the fraction of b_{sp} due to [SO₄²⁻]. Such measurements need to be made in the future, especially resolving [SO₄²⁻] as a function of size.

(6) The cases in which the correlation coefficient was low, and the cases where [SO₄²⁻]/b_{sp} < 0.2 g m⁻² do not fit the simple model of [SO₄²⁻] dominance of b_{sp}. In the case of a ratio greater than 0.2 g m⁻², it seems possible that large particle SO₄²⁻ was present. Further sampling and chemical analyses are needed to explain these cases. There were very few cases of [SO₄²⁻]/b_{sp} < 0.1 g m⁻², so that there was nearly always sufficient SO₄²⁻ to account for the observed b_{sp}. When the SO₄²⁻/b_{sp} was 0.2 g m⁻², assuming a particle size distribution similar to that found by Whitby¹⁶ (log normal, geometric standard deviation = 2, mass mean radius = 0.3 µm) leads to the conclusion that much, and perhaps most, of the light scattering was due to SO₄²⁻. If the measured ratio was ~0.1 g m⁻², then perhaps half of the optical effect can be attributed to SO₄²⁻ (assuming constant size distribution, and so on). Further, the two separate analyses (regression analysis and comparison of measured ratios with those from theory and other experiments) are consistent with this same conclusion.

Since the SO₄²⁻ in southern Scandinavia in the selected meteorological conditions probably arises from the oxidation of man-made SO₂ (ref. 24), these measurements tend to indicate that SO₄²⁻ is not only involved in rain chemistry but also in the aerosol and its optical consequences.

Using this approach, it is interesting to consider the [SO₄²⁻]/b_{sp} ratio as an index of the role of sulphur in tropospheric optics and (since man is rapidly changing the sulphur cycle) also as an index of the effects of human activity on atmospheric optics. Before we can be certain, however, more data will be needed, especially with regard to the non-human, natural sources of submicrometre SO₄²⁻ aerosol. Until such time as these data exist, we feel that the study of the [SO₄²⁻]/b_{sp} ratio and its variations is warranted to explore and test these hypotheses.

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Prevailing winds in lower thermosphere

WINDS in the upper atmosphere have been measured at Adelaide, South Australia (35°S) by radio observations of drifting meteor trails, using a combined continuous wave and pulse technique¹. The systematic features of the winds can be described in terms of a prevailing component and 24-, 12- and 8-h periodic components. Here we are concerned primarily with the behaviour of the prevailing component in the height range 75–105 km for the six years from June 1966 to June 1972.

The observations were usually carried out on four successive days near the middle of each month. Each quarter the observing interval was extended to 7 d, and on a few occasions to 20 d. An average of 250 usable echoes giving drift measurements were recorded each day. The analysis was carried out using a least squares fitting method developed by Groves². For each day this gives the most probable height profiles of the prevailing and periodic wind components. The precision in height is ± 2 km.

Figure 1 shows isotachs of the zonal and meridional winds. The zonal wind has a very regular annual pattern and exhibits two distinct circulation regimes. Below 85 km eastward winds occur in winter and westward winds in early summer, corresponding to the monsoonal type circulation that dominates the air motion in the mesosphere and upper stratosphere. Above 90 km the main features of the circulation are a strong eastward flow in summer, a westward flow in winter and early spring, and relatively large gradients at the solstices. A rapid build-up of eastward flow occurs at upper levels during October and November reaching speeds as high as 80 m s^{-1} at 100 km in December. In some years a second but weaker maximum in the upper circulation pattern occurs about March. The superposition of the two zonal circulation systems produces a complex behaviour near 90 km where the flow is predominantly eastward, and lacks any marked annual variation as illustrated in Table 1.

The reversals of zonal flow that occur at the lower levels are of short duration, typically ~ 1 month. The times of reversals show a phase retardation with height of $0.3\text{--}1 \text{ d km}^{-1}$. On three occasions, during the months of November, 1966, 1969 and 1970 the reversals extended up to 100 km and on two occasions merged with the westward flow of the upper pattern just before its changeover to the strong summer circulation.

Table 1 Amplitudes and phases of the annual and semi-annual waves

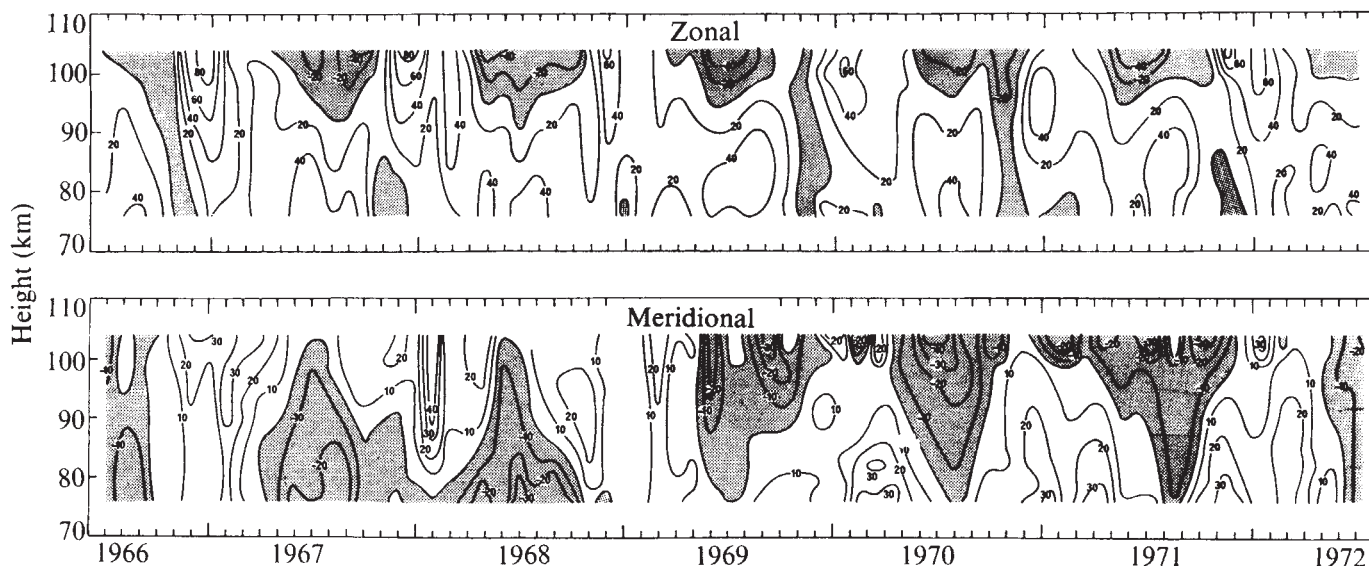
Height (km)	Zonal wind			
	Annual Amplitude (m s^{-1})	Annual Phase (date)	Semi-annual Amplitude (m s^{-1})	Semi-annual Phase (date)
82	16	May 25	7	January 10
90	6	February 21	6	December 26
98	25	December 28	9	December 15

Height (km)	Meridional wind			
	Annual Amplitude (m s^{-1})	Annual Phase (date)	Semi-annual Amplitude (m s^{-1})	Semi-annual Phase (date)
82	14	January 4	4	March 15
90	13	December 28	4	March 6
98	12	December 28	3	February 24

The meridional prevailing winds exhibit annual reversals that are symmetrical in time with respect to the solstices, although the time of a reversal can change with height as rapidly as 7 d km^{-1} . The maximum southward flow occurs about the time of the winter solstice at all heights while the northward flow usually exhibits two maxima close to midsummer. Extreme meridional speeds rarely exceed 30 m s^{-1} . The pattern of the meridional flow changed in early 1969 so that the predominantly northward flow that had occurred at the upper levels since mid-1966 became a southward flow of almost equal strength. This change is reflected in the gradient of the meridional wind as shown in Fig. 2. The gradient values have been calculated at the 90-km level but are generally typical of the whole height range under investigation. The changeover commenced about October 1968 and was completed by April 1969. Early in 1973 a return to positive meridional gradients occurred. There is no evidence of any substantial complementary changes in the zonal flow at these times.

The variability in the meridional gradient during the years 1969–72 is of the order of the mean value of the gradient so that the extremes range between 0 and $-2 \text{ m s}^{-1} \text{ km}^{-1}$. The times of occurrence of the maxima and minima do not show any seasonal dependence. In general the gradients that occur in the meridional flow are much smaller than those in the zonal flow. At 90 km the zonal gradients range from a mean value of $2.2 \text{ m s}^{-1} \text{ km}^{-1}$ in summer to $-3.7 \text{ s}^{-1} \text{ km}^{-1}$ in winter. An extreme value of $-5.5 \text{ m s}^{-1} \text{ km}^{-1}$ occurred during the winter of 1969. Even higher values occur near 100 km.

Fig. 1 Height-time cross sections of the zonal and meridional prevailing winds for the period June 1966–June 1972. The shaded areas represent winds toward the west and toward the south. Speeds in m s^{-1} .



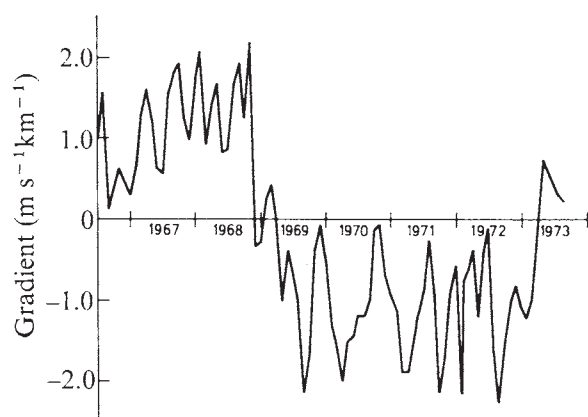


Fig. 2 Vertical gradient of the meridional prevailing wind measured at 90 km showing a sudden change in early 1969.

The annual and semi-annual variation in the prevailing winds have been determined for the period June 1966–June 1972, and the amplitudes and phases (times of maximum eastward or northward flow) at the heights 82, 90 and 98 km, are given in Table 1.

The phase of the annual oscillation in the zonal winds at 82 km is consistent with the phase of this oscillation in the lower mesosphere determined from rocketsonde data¹. These results show that at 35°S and at a height of 65 km, maximum eastward flow occurs in late June and tends to be earlier with increasing height. The amplitude of the annual wave deduced from the rocketsonde data is 60–70 m s⁻¹ at 65 km, whereas the meteor drift observations indicate an amplitude of 16 m s⁻¹ at 82 km. In spite of this rapid fall off in amplitude above 70 km, the consistency of the phase between the heights of 60 and 80 km supports the earlier comment that the mesospheric circumpolar vortex extends to a height of ~ 85 km.

The zonal semi-annual oscillation at 82 km has its maximum eastward flow on January 10; this is about three months earlier than the time of occurrence of maximum eastward flow at 65 km, determined from the rocketsonde data. Such a rapid phase shift with height is not consistent with the relatively slow change in phase below 65 km and requires further investigation.

At a height of 98 km the annual oscillation in the zonal wind has an amplitude of 25 m s⁻¹ and its strength increases with height. The time of maximum eastward flow occurs close to mid-summer. This value of phase is maintained up to the limit of the data at 104 km and is probably typical of this oscillation in the lower thermosphere.

In addition to the annual and semi-annual waves in the zonal winds, oscillations with periods of four months and three months occur with significant amplitudes. These reflect the rapid increase of the zonal wind strength during the months October to December. The meridional prevailing winds are dominated by the annual oscillation whose maximum northward flow occurs almost simultaneously

at all levels, a few days after the summer solstice. It is of interest to note that this annual oscillation was essentially unaffected by the major change in the mean meridional flow that occurred in 1969.

The significance of the mean meridional flow, its long term variations and annual oscillation is open to conjecture. One possibility is that the flow arises from a circumpolar standing wave with a phase variation with height. An annual variation in the phase constant of the wave would account for the annual variation in the meridional winds at any level. Further, a long term change in the phase constant of the whole wave system could reverse the gradient of the winds as is observed. It has been shown⁴ that such a standing wave can transport significant quantities of heat towards or away from the polar regions.

An alternative possibility is that the observed meridional flow is typical of the mean meridional flow across a circle of latitude. This situation has been considered by Ebel⁵ who has shown that such flows imply meridional cells with vertical velocities of the order of a few cm s⁻¹, and momentum sources and sinks of ~ 100 ms⁻¹ d⁻¹ per unit mass. The strength and direction of flow in the cells would undergo annual changes, while long term changes in the observed meridional winds could be produced by changes in the latitude of the cell boundaries. If the latitudinal distribution of the zonal and meridional winds are known, it is also possible to calculate the strengths of the heat and momentum sources required to drive this meridional circulation⁶. The actual situation is likely to be a combination of both mean meridional flow and circumpolar standing waves. The contribution which each makes to the observed north-south winds must await simultaneous observations at several sites around a circle of latitude.

On a limited number of occasions when observations extended continuously for ten days or more, slow and regular changes in the strength of the zonal and meridional winds have been observed. The magnitude of the variation is comparable to the speed of the mean flow and the quasi-period is ~ 10–15 d. This transient motion occurs at all times of the year and is interpreted as planetary waves. The intensity of the variability produced by this long wave activity has been determined by calculating, for each season, the s.d. of the observed daily values of zonal and meridional flow from the mean monthly values. The results are given in Table 2. The strength of the zonal variability is independent of the season at 82 km, although above 90 km it is marginally smaller in summer. The pattern of meridional variability showed a marked change in 1969. For the period 1969–72, its magnitude is essentially independent of season and shows only a marginal increase with height: for the period 1966–69 the variability is two to three times larger in summer than in winter. Although the mechanism for this change in the strength of the variability is not known, it is clearly associated with the reversal of the gradient of the mean meridional flow which occurred in early 1969.

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Table 2 Standard deviations of the zonal and meridional prevailing winds (speeds in m s⁻¹)

Height (km)	Summer	Zonal Autumn	Winter	Spring
82	16	15	14	14
90	12	13	15	18
98	13	17	17	18

Height (km)	Summer	Meridional Autumn	Winter	Spring
82	1966–69	1966–69	1966–69	1966–72
82	27	16	11	11
90	28	12	10	10
98	25	14	15	12

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Mercury in Mersey estuary sediments, and the analytical procedure for total mercury

THE Mersey estuary consists of a wide, shallow upper region, separated from the sea by deeper 'narrows'. The upper estuary (Fig. 1) is an area of continuous accretion of sediment, observed since the mid-nineteenth century with occasional short periods of depletion. The main freshwater inflows are the River Mersey itself, flowing over Howley Weir, and the Manchester Ship Canal, entering the estuary at Eastham Locks. The River Weaver flows into the Man-

and the results presented in Fig. 1 and in Table 1 are compared with other data. The levels found are, in general, somewhat higher than in other, more limited, surveys.

The samples were obtained as follows: the top 5 cm of the sediment were sampled, and 10–20 g were taken at each point. The samples were stored frozen, in glass bottles, until analysis. Total mercury was determined by cold vapour atomic absorption spectrophotometry following digestion in aqua regia, and oxidation with a mixture of potassium dichromate and potassium persulphate^{3,4}. This was found to be superior to the potassium permanganate mixture suggested previously^{3,5}, as this has a tendency to deposit oxides of manganese on the vessel walls, trapping a pro-

Table 1 Mercury concentrations in surface sediments

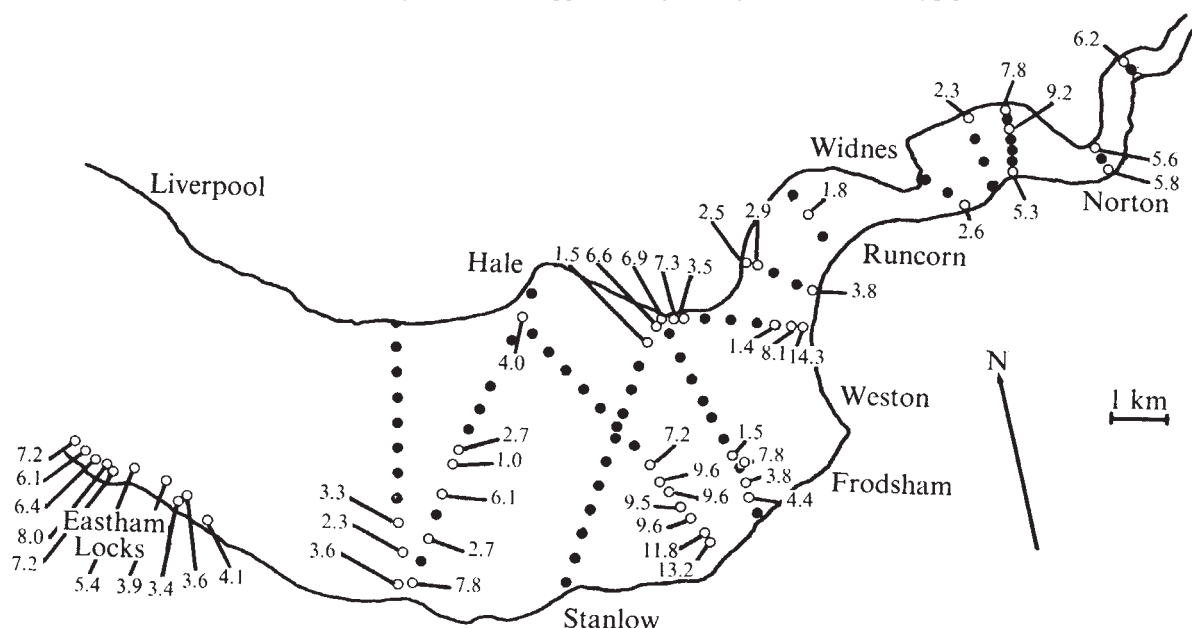
Sample Area	Number of Samples	Total mercury ($\mu\text{g g}^{-1}$)			Reference
		Mean	Min	Max	
River Mersey, England	136	2.23	0.01	14.30	this work
Swansea Bay, Wales	55			1.60	7
Swansea Bay, Wales	11	0.19	0.04	0.76	8
La Have River, Nova Scotia	5	0.34	0.09	1.06	9
Lake Windermere, England	1	1.05			10
Lake Superior	405	0.08		0.58	11
Lake Michigan (South)	31	0.15	0.03	0.38	11
Lake Michigan (North Channel)	55	0.15	0.08	1.11	11
Lake Huron (Georgian Bay)	117	0.26	0.01	9.50	11
Lake St Clair	55	0.63	0.07	2.57	11
Lake Erie	243	0.61	0.13	7.49	11
Lake Ontario	248	0.66	0.03	2.10	11
Pallanza Bay (Lake Maggiore), Italy	34	7.65	0.24	20.10	11
Everglades, Florida	10	0.62	0.12	1.50	12, 13
Mobile Bay	4	0.38	0.22	0.60	12, 13
Mississippi River	4	0.38	0.08	0.57	13
Lake Huleh, Israel	15	1.07			14
Southampton Water, England	3	0.30	0.19	0.64	15*
Thames Estuary, England	15	0.132	0.02	0.49	16

*Mean value including two 10-cm core samples = 1.60 p.p.m.

chester Ship Canal at Weston¹. The area is industrialised and heavily populated and the estuary receives both domestic sewage from surrounding towns and industrial effluent^{1,2}. We are aware of no published data for mercury concentrations in the sediments of the estuary, and have undertaken such measurements. Samples were obtained from the places shown in Fig. 1. We analysed 136 samples for total mercury

portion of the mercury. Replicate analyses of 10 identical test samples gave a mean value of $2.46 \mu\text{g g}^{-1}$, and a r.s.d. of 3.3%. The detection limit (the mean of the blank, plus twice the s.d. of the blank) was $\sim 0.01 \mu\text{g g}^{-1}$ in the sediment. Portions of the samples were dried to determine the water content, and all results are quoted as μg per g of dry sediment.

Fig. 1 Total mercury levels in the upper Mersey estuary. ● indicates $< 1 \mu\text{g g}^{-1}$.



The mean value in the 136 samples was $2.23 \mu\text{g g}^{-1}$. The maximum value recorded was $14.3 \mu\text{g g}^{-1}$, and 23 samples were below the detection limit. High values occurred in those sediments consisting of fine silt, with a large amount of organic material present. This type of sediment is found along the edges of the water course. Lowest values were found in the relatively clean sandy material from the fast running tidal channels of the centre of the estuary. Figure 1 shows Hg concentrations from sampling points with levels $>1 \mu\text{g g}^{-1}$. Sampling points with levels $<1 \mu\text{g g}^{-1}$ are shown as dots. These results indicate three main areas with significantly elevated total mercury concentrations; at Norton, Weston, and Stanlow. Distribution of mercury in the sediments reflects the position and movement of the fine silt in the estuary, and a high mercury level is not necessarily indicative of a mercury input at that point.

Several studies of total mercury concentrations in sediments, but mainly relating to North America, have been carried out and comparison with our work is made in Table 1.

It was not possible to analyse all samples immediately and a report that if a sample of a river sediment containing mercury is taken and stored at -15°C , 50% of the total mercury present originally is lost after 7 d (ref. 6), was therefore relevant to our work. We investigated this alleged loss of mercury from frozen sediments to determine the effect of storage in various conditions on the levels of total mercury found when analysis was carried out.

We found that total mercury does not seem to be lost in significant quantities from samples stored at -20°C ,

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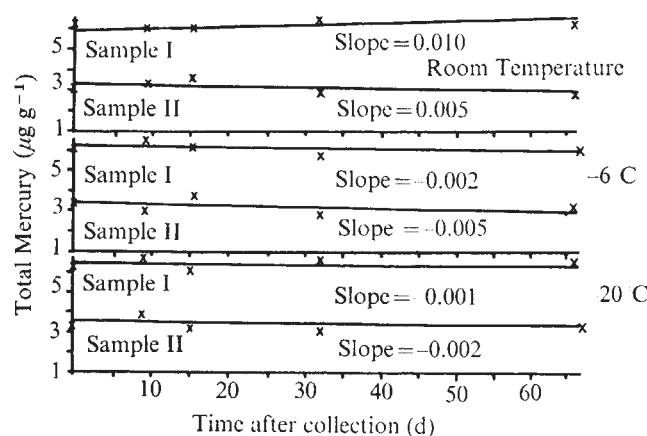


Fig. 2 Repeated analysis of sediment for total mercury

-6°C and even at room temperature (allowing for natural temperature variations). This does not agree with previous work, but a possible explanation is that in Daughdrill's samples⁶ the mercury was present in the form of methyl mercury or that mercuric sulphide was being formed. The form of the sampling was as follows: a bulk sample was taken initially and immediately homogenised and divided into a number of smaller samples. These were frozen with solid carbon dioxide and taken to the laboratory where the first analysis was made the next day (day 0, corresponding to the true environmental level). The data presented in Fig. 2 are of importance for analytical work since they indicate that sediments containing mercury may be stored even at room temperature for at least 9 weeks and still give reliable values for total mercury content.

We acknowledge the assistance of the North West Water Authority, and ICI Brixham Laboratory and Mond Division in this work. Samples were obtained using a hovercraft during a survey by the Brixham Laboratory, or by the

Aliphatic amines in the Murchison meteorite

ANALYSES of the organic compounds found in the Murchison meteorite, a C2 chondrite that fell in Australia in 1969, have provided much new information about extraterrestrial organic chemical evolution. The soluble fraction of this meteorite has been shown to contain aromatic and aliphatic hydrocarbons¹⁻⁴, mono-⁵ and dicarboxylic acids⁶ and various nitrogen-containing organic compounds. The latter include both amino acids^{2,3,7-9} and nitrogen-heterocyclic compounds¹⁰⁻¹¹. The presence of amines, ureas and amides has been suggested¹¹, although no individual compounds from these classes have been identified. Here we report the determination of aliphatic amines in water extracts of the Murchison meteorite.

The Murchison samples used in this work were clean interior specimens obtained from the Center for Meteorite Studies, Arizona State University. All glassware was acid-washed and heated in an oven at the annealing temperature of glass before use. Water for extraction was distilled in an all-glass distilling apparatus from alkaline potassium permanganate. The Murchison fragments were crushed in a clean mortar and transferred to the extraction vessel. One set of samples was extracted in a round bottomed flask under reflux conditions as described previously⁷. These samples were subsequently hydrolysed in 6 N HCl. Another set of Murchison samples was extracted in sealed ampoules at 120°C for 4 d.

The amines of the aqueous extracts were analysed by gas chromatography both as the free amines (Fig. 1a) and as 2,4-dinitrophenyl (DNP) derivatives (Fig. 1b). Methylamine, ethylamine, diethylamine, isopropylamine, *n*-butylamine, *sec*-butylamine, isobutylamine, and *tert*-butylamine were identified as the free amines. Diethylamine and *n*-propylamine could not be identified unambiguously by this method because of incomplete resolution. Peaks appear on the chromatogram at retention times that do not correspond to those of any of the known amines. This is not surprising since the amines were not separated from other organic material in the meteorite extract before chromatography. But most of the interfering low molecular weight compounds would be expected to have been volatilised during reduction in volume of the solution, and other compounds known to be present in the extracts such as fatty acids and dicarboxylic acids should have been trapped as salts in the injection port. Injection of an 18-component amino acid standard solution (Bio Rad Laboratories) did

not cause elution of any peaks corresponding to known amines although a few peaks were apparent that matched unidentified peaks in the Murchison extract.

In order to strengthen the evidence for amines, gas chromatographic separation of the DNP derivatives was carried out¹⁴. In Fig. 1*b* peaks are seen corresponding to the DNP derivatives of ethylamine, dimethylamine and diethylamine, as well as at the positions of the unresolved pairs, isopropylamine-*tert*-butylamine, methylamine-*sec*-butylamine, and *n*-propylamine-isobutylamine. A large dinitroaniline (DNA) peak is observed in addition to several unknowns.

Primary amines can be readily determined with an amino acid analyser by extending the final elution step (J.R.C. and P. E. Hare, unpublished). This method was used for analysis of the Murchison amines and the chromatogram is shown in Fig. 2. Well resolved peaks are seen corresponding to methylamine, ethylamine, isopropylamine, *tert*-butylamine, *n*-propylamine and *n*-butylamine. An unresolved peak is seen corresponding to *sec*-butylamine and/or isobutylamine. Secondary amines are not detected with this system as a result of their lack of reactivity with the detection reagent. An aqueous extract of the meteorite that had not been subjected to acid hydrolysis was also chromatographed. The amounts of each amine found, both before and after acid hydrolysis, are given in Table 1.

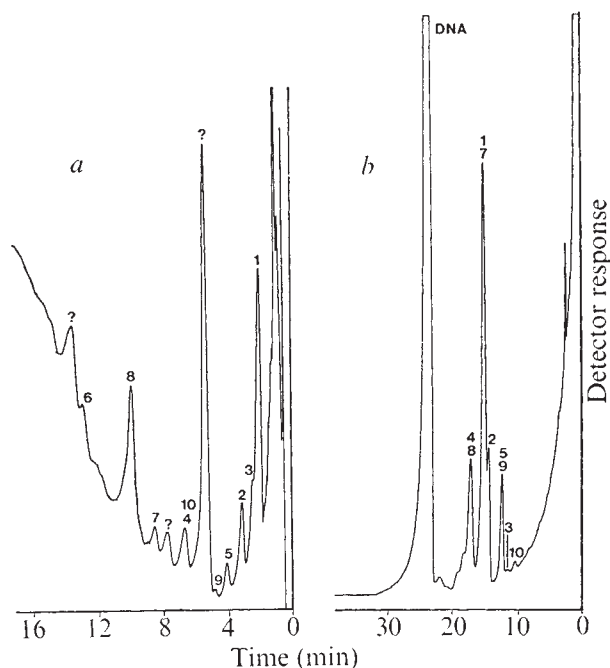


Fig. 1 Gas chromatographic analysis of meteorite amines. The peaks corresponding to known amines or DNP amines are identified by number as in Table 1. *a*, Gas chromatography of the free amines. An extract derived from approximately 0.5 g of the Murchison meteorite was acidified and evaporated to near dryness with gentle heating under a stream of purified nitrogen. The resulting solution was injected directly into the injection port of a Perkin-Elmer 3920 gas chromatograph. The injection port was packed with 25% potassium hydroxide on 80/100 mesh Chromosorb W in order to liberate the free amines from the hydrochloride salts¹². The gas chromatograph contained a 12 foot $\times$ 1/8 inch stainless steel column packed with 20% UCON LB 550-X plus 10% KOH on 120/140 mesh Chromosorb W and was temperature programmed from 60 °C to 110 °C at 4 °C min⁻¹ with a helium flow rate of 50 ml min⁻¹. *b*, Gas chromatography of the DNP amines. The amine DNP derivatives were prepared from 2 ml of meteorite extract (0.8 g meteorite) by reaction with sodium dinitrobenzenesulphonate¹³. The reaction time was lengthened to enhance the formation of DNP derivatives of the secondary amines. The DNP amines were extracted into benzene and washed with a saturated borax solution. The volume of the benzene layer was reduced to a few μ l and analysed on a 6 foot $\times$ 1/8 inch stainless steel column packed with 7% XE-60 on 110/120 Anakrom ABS.

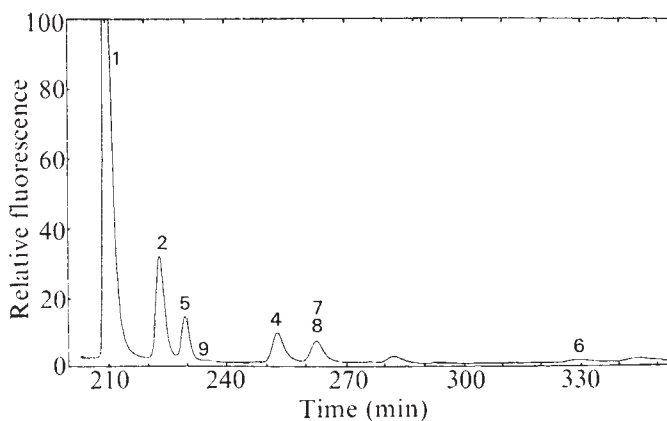


Fig. 2 Ion exchange chromatographic analysis of meteorite amines. The peaks corresponding to known amines are identified by number as in Table 1. An acid-hydrolysed water extract from 0.09 g Murchison meteorite was applied to a 0.20 $\times$ 25 cm DC4A resin (Durrum Chemical Co., Palo Alto) column. Separation of amino acids in the sample was achieved by sequential elution of the column with citrate buffers (0.2 M sodium ion) of increasing pH. Basic amino acids, followed by amines, were brought off the column by continuing the elution with a 0.032 M borate buffer, pH 10.7, which had been brought to 0.23 M in sodium ion by the addition of NaCl. Amino acids and amines were detected fluorometrically after reaction with *o*-phthalaldehyde¹⁵. Only the latter part of the overall chromatogram is reproduced above.

The results summarised in Table 1 give evidence for the presence in water extracts of the Murchison meteorite of all of the possible primary aliphatic monoamines (eight) with fewer than five carbon atoms. Five of these amines could be identified unambiguously in at least two of the three chromatographic systems used. Two of the seven possible secondary or tertiary aliphatic monoamines were identified. Chromatographic standards were lacking for the remaining five amines of this type and they went unrecognised, if present.

The primary amines that could be identified in the aqueous extract total 80 nmol per g meteorite. It is interesting to note that these compounds, some of which are quite volatile, have not been lost during the travel of the meteorite in space. They thus seem to be chemically or physically trapped in the meteorite.

Two similarities are apparent between the water-extractable amines and amino acids of the Murchison meteorite: (1) The level at which amines are present declines sharply with increasing carbon number in the homologous series, methylamine, ethylamine, *n*-propylamine, *n*-butylamine. Although the decline in content is not as sharp, the same is true within the amino acid series,

Table 1 Chromatographic identification of amines

Amine	Gas chromatography		Ion exchange chromatography	
	Free amine	DNP amine	Extract	Hydrolysed extract
1 Methylamine	+	+	49	143
2 Ethylamine	+	+	14	30
3 Dimethylamine	+	+	†	†
4 <i>n</i> -Propylamine	+	+	5	10
5 Isopropylamine	+	+	9	13
6 <i>n</i> -Butylamine	+	+	0	3
7 <i>sec</i> -Butylamine	+	+	+	+
8 Isobutylamine	+	+	+	+
9 <i>tert</i> -Butylamine	+	+	3	3
10 Diethylamine	+	+	†	†

*Unambiguous identification not possible because of incomplete resolution.

†Secondary amines not detectable with *o*-phthalaldehyde.

glycine, alanine, α -amino-*n*-butyric acid, norvaline, norleucine. These quantitative relationships are consistent with a chemical synthesis in which a simple carbon compound, methane, for example, is the precursor of meteorite amines and amino acids. (2) The amine content of the aqueous meteorite extract increases by over 100% on acid hydrolysis. A similar observation has been made with respect to the amino acids in aqueous extracts of both the Murchison and Murray meteorites⁷. Thus both amines and amino acids are extracted from the meteorite as such, as well as in the form of acid-hydrolysable derivative or precursor species.

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Antiquity of man in America indicated by radiometric dates on the Yuha burial site

MUCH evidence suggests that man was present in the Western Hemisphere before 12,000 yr ago, but the case has remained less than conclusive¹. In some situations, the geological age of the site is reasonably well established but the association or nature of the artefacts is questionable^{2,3}. In other cases, museum specimens of human bones dated by radiocarbon analysis of collagen lack desirable information concerning site location, geology, and stratigraphy even though the accuracy of their absolute ages seems valid⁴⁻⁶. We report here the results of radiometric dates of the Yuha burial site from Imperial County, California, for which the geology and stratigraphy have been documented and reported in detail⁷.

Claims of great antiquity on human bones based on amino acid racemisation⁸ also suffer from shortcomings. The applicability of this technique by itself to bones where burial history is unknown is doubtful, and successful application of this technique requires a known temperature history of the bone, or, alternatively, a radiocarbon calibration point⁹⁻¹³.

The Yuha site was discovered and excavated in 1971 and consisted of a partially preserved skeleton buried 35 cm

below the surface. The burial was located on a dissected alluvial fan at the edge of a now dry pluvial lake, and was covered by a cairn of local cobbles and boulders. Flaked stone artefacts of a unifacial lower lithic industry were associated with the skeleton⁷. Subsequent to the time of burial, soil caliche precipitated, cementing the upper 15-60 cm of the entire alluvial deposit, coating the bones, the buried cairn boulders, and the underside of the exposed boulders.

A sample of the caliche coating the underside of one of the exposed cairn boulders (sample 103) and a sample of caliche coating one of the bone fragments (sample 104) were chosen for radiocarbon analysis. The results indicate ages of $22,125 \pm 400$ yr BP for sample 103 (R.P., UCLA2600) and $21,500 \pm 1,000$ yr BP for 104 (Geochron Laboratories, Cambridge, Massachusetts, GX2674). A modified ²³⁰Th dating technique for application to soil caliches has been developed (T. L. Ku, W. B. Bull and K. G. Knauss, unpublished), but the technique can be successful only when corrections can be made for the detrital impurities. This was possible only for sample 103, which yielded an absolute age of $19,000 \pm 3,000$ yr BP, in good agreement with the radiocarbon results. In as much as the details of the ²³⁰Th technique are yet to be published, however, the case for the antiquity of the Yuha skeleton must rest heavily on the radiocarbon results.

In proper conditions and with careful sample selection radiocarbon dates on soil caliches from arid zone palaeosols can indicate the true age of soil formation^{14,15}. The Mungo burial in Australia represents essentially the same arid zone setting, environment, and time frame as the Yuha burial. Radiocarbon dates of soil caliche associated with this burial reflect the true age of local caliche pedogenesis¹⁶.

A lone radiocarbon date on soil caliche, however, is always suspect because of possible contamination by younger organic matter in the soil or by older or 'dead' carbon from detrital limestone. Random or scattered apparent ages would result from such contamination, however, if more than one sample were analysed from a small area, since contamination is unlikely to be homogeneous over any distance. The close agreement among our samples with respect to age strongly suggests they are free from such contamination and represent the true age of the caliche precipitation. The ²³⁰Th date supports this conclusion.

These results compare favourably with a date of $2,4000 \pm 1,000$ yr BP (sample 401, ref. 7 and Geochron GX2722) obtained on soil caliche at the same elevation on the alluvial fan (134 m above sea level, but at a distance of 2 km from the burial). Similar caliches taken at successive distances up the slope from the burial and at higher elevations in the fan (250-300 m) yield successively older ages of 27,000-30,000 yr BP (ref. 7), indicating that caliche pedogenesis was progressive, beginning at the head of the fan and progressing down slope as the water table receded in response to desiccation of the pluvial lake. The original base level of the fan itself was apparently the high stand of the pluvial lake, at 45 m elevation, dated by lake shore tufa and freshwater snails at 37,400 yr BP (ref. 7).

In as much as the time of burial predates the precipitation of local caliche, our results yield a minimum age for the Yuha burial. Absolute dating of the skeleton itself by the collagen-radiocarbon technique will be carried out, but will require destruction of most of the skeletal material, since preliminary microanalytical analyses yielded a nitrogen content of only 0.68%. Consequently, such dating must await the completion of physical anthropological studies and construction of casts of each bone. With two A₂ dates the absolute chronological position of the Yuha hominid around 22,000 yr BP or older seems to be positively affirmed¹⁷.

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Estimates of speeds of dinosaurs

THE faster an animal walks or runs the longer, in general, are its strides. Many dinosaur tracks have been found from which stride lengths can be measured¹. I have now obtained a relationship between speed, stride length and body size from observations of living animals and applied this to dinosaurs to achieve estimates of their speeds. The estimated speeds are rather low—between 1.0 and 3.6 m s⁻¹.

Estimates of the speeds of large dinosaurs must depend on data from smaller animals. A means of allowing for differences in size is offered by the theory of physical similarity².

If meaningful comparisons are to be made between animals of different sizes, an appropriate non-dimensional parameter is needed to serve as a criterion for physical similarity. The most familiar parameter of this type is the Reynolds number which applies in situations where inertia and viscous forces interact and is very important in aerodynamics. Less familiar to biologists is the Froude number, which applies to any situation where inertia and gravity interact, and is important in nautical engineering. The Froude number is u^2/gl where u is the velocity, g the acceleration of free fall and l a characteristic length (in nautical engineering, the hull length) which, in this application to terrestrial locomotion, is the height h of the hip from the ground. The Froude number then becomes u^2/gh .

Considerations of physical similarity predict that the movements of animals of geometrically similar form but of different sizes will be geometrically similar only when they move with the same Froude number u^2/gh (that is, when the squares of their speeds are proportional to their linear dimensions). Geometrically similar movements require equal values of λ/h (that is, their stride lengths also must be proportional to their linear dimensions). The theory of physical similarity further predicts that, even when the Froude numbers are not the same, λ/h will be a function of the Froude number, although this function is not defined; we are obliged to obtain this relationship by empirical methods. It should be noted that stride length λ in this paper means the distance between corresponding

points on successive prints of the same foot³; the term is used to mean half this distance in some accounts of dinosaur tracks.

Animals of different sizes are not geometrically similar; there seems, however, to be a rather widespread relationship between λ/h and u^2/gh (ref. 4). Figure 1a shows that for mammals as diverse as jirds, men and horses

$$\lambda/h \approx 2.3(u^2/gh)^{0.3} \quad (1)$$

Figure 1b shows that for man, and presumably for other animals, the relationship between stride length and speed is not appreciably different for firm ground and soft mud. Equation (1) can be written

$$u \approx 0.25g^{0.5}\lambda^{1.67}h^{-1.17} \quad (2)$$

so that u can be estimated from known values of λ and h . λ can be measured accurately from tracks but h can only be estimated from the size of the footprints (see below). Equation 2 shows that if h is overestimated by 10%, u will be underestimated by 11%.

The tracks to be considered were made by unidentified bipedal dinosaurs and sauropods. It is assumed in each case that the metatarsophalangeal joint rested on the ground, but the tarsometatarsal joint did not. If this is correct, the length of the hind footprint is 0.23–0.28 h for many bipedal dinosaurs of a wide range of sizes, both Theropoda and Ornithopoda, and about 0.25 h for *Apatosaurus* (Sauropoda) (see for instance the photographs of skeletons in refs 5 and 6). It will therefore be assumed that hind footprint length is 0.25 h in all cases.

All the data in Table 1 refer to trails of fairly evenly spaced footprints, indicating progression at fairly constant speed. The plan in ref. 7 from which some of the data are taken has

Fig. 1 Graphs on logarithmic coordinates of relative stride length (λ/h) against Froude number (u^2/gh). *a*, Data from travelling on firm ground or treadmills for men^{10,11} walking and running, horses¹², jirds (*Meriones unguiculatus*, unpublished observations of S. N. G. Frodsham and R. McN. A.), elephant¹² (*Elephas maximus*) (●) and ostrich¹² (*Struthio camelus*) (○). *b*, New data for man walking and running on hard ground (○), sandy mud (●) and soft mud (●) at Snettisham beach, Norfolk. The subjects were an adult man and, two children (aged 11 and 13), each of whom made 4–8 walks or runs at various speeds (in random order) over a 25 m course on each substrate. They made moderate footprints in the sandy mud and very deep ones in the soft mud, on which it was very difficult to run. The broken line on each graph is

$$\lambda/h = 2.3(u^2/gh)^{0.3}.$$

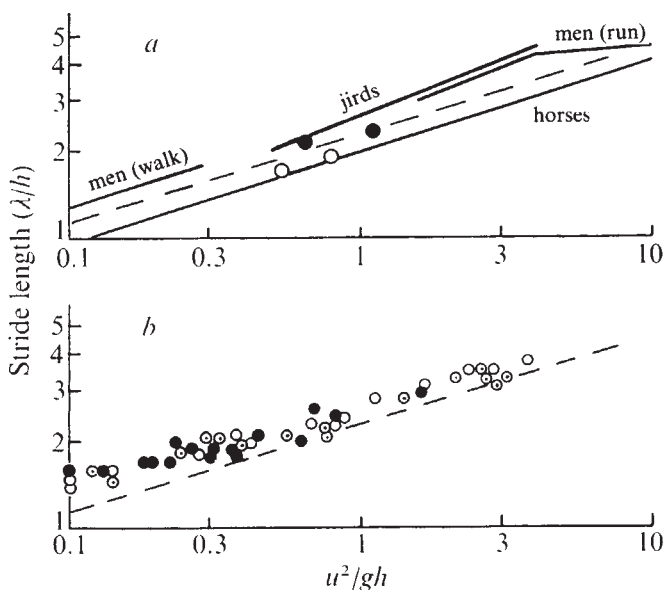


Table 1 Dimensions of dinosaur tracks and data estimated from them

Reference	Bipedal dinosaurs						Sauropods	
	13	7	7	14	1	1	7	7
Stride length, λ (m)	3.0	3.0	2.4	1.3	1.8–2.5	1.5–1.8	2.5	1.6
Hind foot length (m)	0.53	0.50	0.24	0.27*	0.28–0.35	0.15–0.20	0.76	0.38
Hip height, h (m)	2.1	2.0	1.0	1.1	1.1–1.4	0.6–0.8	3.0	1.5
Relative stride length, λ/h	1.4	1.5	2.5	1.2	1.7	2.4	0.8	1.1
Speed, u (m s ⁻¹)	2.0	2.2	3.6	1.2	2.2	2.9	1.0	1.1

*Footprint length taken from measurements at the British Museum (Natural History). Other dimensions are taken from the published descriptions.

no scale but the paper includes a photograph of part of the same area marked out in yard squares, from which the scale has been determined. The speeds are rather low, especially those of the sauropods. Large and small sauropods seem to have been walking at the same speed, consistent with the suggestion that they were travelling as a herd^{11,7}.

Mammals generally change from a walk to a run or trot when the Froude number reaches about 0.6 and λ/h reaches 2.0 (ref. 4). Of the tracks recorded in Table 1, only the two fastest seem to show running, and both were made by fairly small dinosaurs. This does not necessarily mean that large dinosaurs never ran, but it seems to conform better with the traditional image of lumbering dinosaurs than with that of the lively runners shown in some recent restorations^{8,9}.

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Stereoscopic vision within the schizochroal eye of trilobites

MOST trilobites had holochroal eyes which were probably analogous with the compound eyes of most living arthropods¹. Trilobites of the suborder Phacopina had schizochroal eyes, in which comparatively few large separate lenses are distributed over the eye surface. Clarkson and Levi-Setti² showed that the schizochroal eye is unique in structure, in that each lens is biconvex and is made of two calcitic elements of different refractive indices, separated by an aspherical surface with a configuration such that the lens was corrected for spherical aberration. A modified interpretation by Campbell³ suggests that the e and o rays were transmitted through the calcite lens to focus in the same plane. Since chromatic aberration is not important in seawater more than a few metres deep, either interpretation implies that each lens of the schizochroal eye was capable of forming an accurate image of its field of vision^{2,3}.

We suggest that the schizochroal eye was adapted to give

stereoscopic vision by the comparison and integration of optically accurate images from lenses within the same eye. Stereoscopic vision depends on the simultaneous perception of the same field of view through two lenses set at some distance apart. The images produced by the lenses should be of sufficient optical quality to define objects at useful resolution, the images should overlap considerably and there should be appropriate neural structures to interpret the information provided by the overlapping images. Towe⁴, Clarkson and Levi-Setti² and Campbell³ have already established the optical quality of the lenses. It remains to be shown that there would have been useful overlap between the images produced by adjacent lenses, and that it is reasonable to infer the existence of a suitable neural network behind the eye.

Clarkson^{5,6} has measured the angular separation between the axes of the lens on many schizochroal eyes. The vertical spacing between lens axes is typically 2–10°, whereas the horizontal spacing is typically 7–20°. Since no part of the visual field is left unscanned, the cone of useful vision of a typical schizochroal lens would have been about 15–20° (for comparison, the ommatidia of *Limulus* have a useful range within 10–20° of their optic axes⁷).

The approximate hexagonal packing of lenses on the schizochroal eye usually included files of lenses arranged dorso-ventrally. Coupled with the astigmatism of the eye surface, this meant that the dorso-ventral files of lens axes formed “visual strips”—files of lens axes with low angular separation, running nearly vertically across the visual field, each file of lenses separated by a significant angle from the next file. If each lens had an effective visual field of 15–20°, the visual fields of neighbouring lenses on each visual strip would have overlapped greatly, whereas the visual field of any given lens would only have overlapped marginally with its nearest neighbour on the next visual strip. Thus, given appropriate neural integration, the potential for stereoscopic vision was very great within each visual strip, but must have been marginal between strips.

We suggest that the large horizontal spacing between visual strips was such as to ensure that the whole visual field of the eye was scanned adequately, while the close vertical separation between lens axes along the visual strips allowed stereoscopic scrutiny over the whole visual field. Some phacopids had eyes in which the angular separation between lenses on visual strips approached the separation between strips. The arguments developed here would comfortably include these species, since the angles involved are never more than 6–7° (ref. 6), and would have permitted stereoscopic integration between lenses on the strips.

Rudwick and Clarkson⁸ have shown how a trilobite might have detected an approaching object as it appeared successively on images produced by different lenses. In the ideal case, any array of closely spaced lens axes—as in a holochroal eye—would have been able to detect approaching objects with equal sensitivity. But in the real world of the trilobite, stereoscopic scrutiny by cooperating lenses arranged in files would more accurately have detected the

existence, size, speed and character of objects in the field of view.

Stockton and Cowen (unpublished) have constructed a simple neurophysiological model of the integration of visual images within a linear array of lenses. According to this model, it is reasonable to suppose that a trilobite with schizochroal eyes could have had the neural apparatus to perceive objects stereoscopically. The model includes predictions of features of morphology found among phacopid trilobites, and it is amenable for testing by further study of the optics of phacopid lenses.

Phacopid trilobites had visual fields that usually extended through 360° in the horizontal plane, but only from 0 to 40° at most above the horizontal. If our suggestion is correct, they would have had stereoscopic vision throughout that field. In fact, they would have had a wider range of stereoscopic vision than any organism that has ever lived.

The system was essentially a defensive one. Hunting predators have stereoscopy which is best developed in a limited anterior field. Prey species often have all-round vision, but usually have limited stereoscopic capability. Phacopid trilobites can thus be interpreted as benthic animals with special adaptations for detecting moving enemies on the sea floor in the photic zone. Cephalopods, arthropods and (in the Devonian) fish would have been likely predators on phacopids.

The Phacopina became extinct at the end of the Devonian period, long before trilobites as a class became extinct. Although less than 10% of Ordovician trilobite genera were Phacopina, however, their representation rose to 10–20% during the Silurian and reached 20–40% during the Devonian⁸. Trilobites with schizochroal eyes were relatively successful for 150 Myr. Nothing like the schizochroal eye has ever re-evolved among the hundreds of thousands of species of later arthropods.

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Parent-offspring resemblance for specific cognitive abilities in two ethnic groups

A LARGE scale study of familial factors in mental ability is in progress in Hawaii and data are being obtained on 15 cognitive variables (Table 1), various environmental indices, and blood group and enzyme systems. After three years of testing, there are now sufficient data (1,490 families; 5,077 individuals) for preliminary genetic analyses. Measures of parent-offspring resemblance for members of the two largest ethnic groups in our sample, Americans of European ancestry (AEA; 739 families) and Americans of Japanese ancestry (AJA; 244 families), are presented here. These measures provide upper-bound estimates for the heritability of performance on the individual tests and factor scores^{1,2}, in addition to a test of the hypothesis^{3,4} that spatial visualisation ability is influenced by a sex-linked major gene. Relatively small scale studies of parent-offspring resemblance for specific cognitive abilities have been reported for a Caucasian sample⁵ and a Korean sample⁶; however, to the

best of our knowledge, this is the first comparative study of parent-offspring resemblance for measures of cognitive abilities in two ethnic groups.

A 'family', for our purposes, consists of both biological parents, 60 yr of age or younger, and one or more of their offspring, 13 yr of age or older. Because of the presence of marked age effects², age correction was deemed necessary; such correction was accomplished by using a z-score banding technique. Age bands consisted of yearly intervals for the ages 15–20 and 34–55, and of 2–6-yr intervals for other ages (depending on sample size).

In the absence of an environmental correlation between relatives, the regression of offspring's score on mid-parental score provides a direct estimate of heritability in its 'narrow sense', that is, the proportion of the observed phenotypic variance due to the average effects of the genes⁷. This method for estimating heritability has several advantages over other methods, including its insensitivity to the effects of non-random mating. (Assortative marriage for specific cognitive abilities in this study has been discussed elsewhere⁸.) For at least some measures of human mental ability, however, environmental correlations between family members are likely to be non-zero and positive. The regressions of offspring on mid-parental values presented here should, therefore, be regarded as measures of phenotypic (genetic and/or environmental) similarity and only upper-bound estimates of heritability.

The regression coefficients for each cognitive test and for five factor scores are presented in Table 2. Two sets of coefficients are given, one uncorrected and one corrected for test reliability. It is important to be clear about when and when not to adjust for lack of perfect test reliability⁹: when estimates are to be used in prediction equations with individual scores (for example, to predict response to selection), uncorrected estimates must be used, but when estimates obtained from tests with different reliabilities are to be compared, correction is appropriate. Since considerable variation can be seen among the reliabilities presented in Table 1, only the corrected regression coefficients will be examined for evidence of differential heritability.

Standard errors for the corrected regression coefficients in Table 2 are sufficiently small, especially for AEA, to indicate that at least some of the variability among estimates is real. An indication of the reliability of the differential estimates among tests is the marked congruence between ethnic groups. The correlation between AEA and AJA coefficients across the 15 individual tests is highly significant (Spearman rank correlation = 0.77, $P < 0.01$). With regard to the four varimax rotated factor scores, the regressions are moderately high for verbal and spatial ability, but somewhat lower for visual memory and perceptual speed in both ethnic groups. These results are highly consistent with previous evidence¹⁰ for the differential heritability of specific cognitive abilities (especially for verbal ability and memory) and afford an opportunity for identification of those environmental factors that differentially affect cognitive abilities.

Our tests also provide a measure of 'general intelligence'. The progressive matrices test is a modified form of the well known test of abstract reasoning developed by Raven¹¹, a 'culture-fair' test which is regarded by many as being one of the best single measures of general intelligence. Our modified form is reduced in length to 30 items (20-min time limit in group administration), but retains a relatively high reliability (see Table 1). Regression of offspring on mid-parental value (uncorrected) for this test is 0.51 for AEA subjects and 0.27 for AJA. These results are consistent with those of Guttman¹², who found the correlation between child and mid-parent in a sample of 100 families in Israel to be 0.41 for the complete test.

The unrotated first-principal component score may also be used as a measure of general intelligence. The regressions of offspring on mid-parental values (uncorrected) for this measure

Table 1 Cognitive tests, test times and reliabilities for two ethnic groups: Americans of European ancestry and Americans of Japanese ancestry

Test	Cognitive factor*	Test time	Reliability†
Primary mental abilities (PMA) vocabulary	V	3 min	0.96
Visual memory (immediate)	M	1-min exposure/1-min recall	0.58
Things (a fluency test)	V	2 parts/3 min each	0.74
Sheppard-Metzler mental rotations (modified for group testing by Vandenberg)	S	10 min	0.88
Subtraction and multiplication	P	2 parts/2 min each	0.96
Elithorn mazes ("lines and dots"), shortened form	S	5 min	0.89
Educational testing service (ETS) word beginnings and endings	V	2 parts/3 min each	0.71
ETS card rotations	S	2 parts/3 min each	0.88
Visual memory (delayed recall)	M	1 min	0.62
PMA pedigree (a reasoning test)	V,S,P	4 min	0.72
ETS hidden patterns	S	2 parts/2 min each	0.92
Paper form board	S	3 min	0.84
ETS number comparisons	P	2 parts/1.5 min each	0.81
Whiteman test of social perception	V	10 min	0.69
Raven's progressive matrices, modified form	S	20 min	0.86

*Varimax rotated factor loading ≥ 0.40 (V, verbal factor; S, spatial factor; P, perceptual speed factor; M, visual memory factor).

†From Wilson *et al.*². Details concerning the testing procedure and factor analyses are available in this source.

are 0.61 and 0.42 for AEA and AJA, respectively. These values are comparable with those obtained by Williams⁵, who reported that the regression of offspring on mid-parent was 0.46 for total IQ as measured by the Wechsler Intelligence Scales. Since the regression of offspring on mid-parent may only be regarded as an upper-bound estimate of heritability, these results suggest that the narrow-sense heritability for tests of general intelligence may be lower than the value of 0.71 reported by Jinks and Fulker¹³ in their re-analysis of previously published data.

Scarr-Salapatek¹⁴ concluded from twin data that the heritability of scholastic aptitudes may differ in different racial groups as a function of socioeconomic status. Although her conclusions have been criticised¹⁵ on the basis of statistical considerations, she has recently¹⁶ reported additional evidence for such a difference. Even though the educational and occupational levels of the two ethnic groups included in the present study are roughly comparable, differences in parent-offspring resemblance may exist between groups. From Table 2 it can

be seen that 14 of the 15 regression coefficients for the individual tests are higher for AEA subjects. Only two of these differences however, are significant. When these data were examined for differences in parent-offspring resemblance as a function of socioeconomic status within ethnic groups, no significant differences were observed.

Single-parent, single-child correlations were also estimated for the individual tests and factor scores in order to explore the possibility of sex-linked inheritance. Stafford³ first reported a pattern of parent-offspring correlations which suggested that spatial visualisation ability may be influenced by a sex-linked major gene. The expected pattern of correlations (r) for such a character is as follows

$$r_{FS} < r_{MD} < r_{MS} = r_{FD}$$

where F, M, S and D are father, mother, son and daughter, respectively. Stafford's original finding has been given considerable support by the more recent report of Bock and

Table 2 Regression of mid-child on mid-parent ($\pm$ s.e.) for cognitive tests and factor scores in two ethnic groups

Tests	Uncorrected		Corrected†	
	AEA	AJA	AEA	AJA
Vocabulary	0.65 $\pm$ 0.05	0.57 $\pm$ 0.07	0.68 $\pm$ 0.04	0.59 $\pm$ 0.07
Visual memory (immediate)	0.15 $\pm$ 0.05	0.16 $\pm$ 0.08	0.26 $\pm$ 0.05	0.28 $\pm$ 0.08
Things	0.41 $\pm$ 0.05	0.27 $\pm$ 0.09	0.55 $\pm$ 0.04	0.36 $\pm$ 0.09
Mental rotations	0.42 $\pm$ 0.05	0.37 $\pm$ 0.09	0.48 $\pm$ 0.05	0.42 $\pm$ 0.09
Subtraction and multiplication	0.38 $\pm$ 0.04	0.29 $\pm$ 0.09	0.40 $\pm$ 0.04	0.30 $\pm$ 0.09
"Lines and dots" (Elithorn mazes)	0.22 $\pm$ 0.05	0.19 $\pm$ 0.08	0.25 $\pm$ 0.05	0.21 $\pm$ 0.08
Word beginnings and endings	0.40 $\pm$ 0.04	0.39 $\pm$ 0.09	0.56 $\pm$ 0.04	0.55 $\pm$ 0.08
Card rotations*	0.46 $\pm$ 0.05	0.25 $\pm$ 0.08	0.53 $\pm$ 0.04	0.28 $\pm$ 0.07
Visual memory (delayed)	0.33 $\pm$ 0.05	0.22 $\pm$ 0.09	0.53 $\pm$ 0.04	0.35 $\pm$ 0.09
Pedigrees	0.53 $\pm$ 0.04	0.46 $\pm$ 0.08	0.74 $\pm$ 0.04	0.64 $\pm$ 0.07
Hidden patterns	0.43 $\pm$ 0.05	0.27 $\pm$ 0.08	0.47 $\pm$ 0.05	0.29 $\pm$ 0.08
Paper form board	0.53 $\pm$ 0.04	0.48 $\pm$ 0.09	0.63 $\pm$ 0.04	0.57 $\pm$ 0.09
Number comparisons	0.39 $\pm$ 0.05	0.32 $\pm$ 0.08	0.48 $\pm$ 0.04	0.40 $\pm$ 0.08
Social perception	0.27 $\pm$ 0.05	0.24 $\pm$ 0.07	0.39 $\pm$ 0.05	0.35 $\pm$ 0.07
Progressive matrices*	0.51 $\pm$ 0.04	0.27 $\pm$ 0.07	0.59 $\pm$ 0.04	0.31 $\pm$ 0.07
Factors				
Verbal	0.57 $\pm$ 0.04	0.51 $\pm$ 0.07	0.65 $\pm$ 0.04	0.58 $\pm$ 0.07
Spatial	0.57 $\pm$ 0.05	0.41 $\pm$ 0.08	0.61 $\pm$ 0.04	0.44 $\pm$ 0.08
Perceptual speed	0.41 $\pm$ 0.05	0.29 $\pm$ 0.09	0.46 $\pm$ 0.04	0.32 $\pm$ 0.09
Visual memory	0.32 $\pm$ 0.05	0.22 $\pm$ 0.09	0.44 $\pm$ 0.05	0.31 $\pm$ 0.09
First principal component*	0.61 $\pm$ 0.04	0.42 $\pm$ 0.07	0.63 $\pm$ 0.04	0.43 $\pm$ 0.06
No. of families‡	739	244	739	244

*Significant difference between AEA and AJA regression coefficients ($P < 0.05$).

†Corrected for test reliability.

‡AEA data from 482 one-child families, 224 two-child families, 30 three-child families and 3 four-child families. AJA data from 198 one-child families, 36 two-child families, 9 three-child families and 1 four-child family.

Table 3 Single-parent, single-child correlations for cognitive tests and factor scores in two ethnic groups*

	AEA				AJA			
	Father-son	Mother-daughter	Mother-son	Father-daughter	Father-son	Mother-daughter	Mother-son	Father-daughter
Tests								
Vocabulary	0.35	0.37	0.36	0.30	0.35	0.31	0.38	0.19
Visual memory (immediate)	0.05	0.10	0.06	0.08	0.06	0.06	0.06	0.23
Things	0.17	0.25	0.25	0.18	0.24	0.14	0.12	0.16
Mental rotations	0.15	0.32	0.16	0.23	0.30	0.10	0.14	0.34
Subtraction multiplication	0.28	0.25	0.17	0.17	0.18	0.14	0.19	0.04
"Lines and dots" (Elithorn mazes)	0.07	0.17	0.17	0.07	0.08	0.04	0.14	0.19
Word beginnings and endings	0.23	0.27	0.24	0.26	0.20	0.14	0.25	0.22
Card rotations	0.26	0.36	0.19	0.22	0.26	0.09	0.12	0.11
Visual memory (delayed)	0.16	0.10	0.14	0.21	0.18	0.10	0.10	0.11
Pedigrees	0.26	0.38	0.32	0.26	0.23	0.31	0.22	0.28
Hidden patterns	0.21	0.32	0.20	0.27	0.10	0.18	0.12	0.26
Paper form board	0.27	0.36	0.30	0.40	0.24	0.24	0.26	0.21
Number comparisons	0.25	0.27	0.24	0.17	0.25	0.09	0.22	0.36
Social perception	0.11	0.15	0.18	0.12	0.09	0.21	0.07	0.26
Progressive matrices	0.20	0.29	0.38	0.27	0.10	0.17	0.22	0.26
Factors								
Verbal	0.27	0.30	0.40	0.31	0.36	0.29	0.35	0.24
Spatial	0.27	0.41	0.28	0.32	0.33	0.13	0.21	0.31
Perceptual speed	0.28	0.27	0.22	0.18	0.23	0.04	0.22	0.20
Visual memory	0.15	0.13	0.13	0.18	0.12	0.10	0.09	0.20
First principal component	0.29	0.45	0.41	0.36	0.30	0.28	0.29	0.30
No. of pairs	434	438	434	438	130	138	130	138

*Correlations greater than 0.10 are significantly different from zero ($P \leq 0.05$) for AEA subjects, whereas those greater than 0.17 are significant for AJA subjects.

Kolakowski⁴. Single-parent, single-child correlations obtained from the data of the present study are presented in Table 3. Of the correlations involving individual tests of spatial ability (mental rotations, "lines and dots", card rotations, hidden patterns, paper form board, progressive matrices), only the set for progressive matrices for AJA subjects conforms to the order expected of a character influenced by a sex-linked major gene. An hypothesis of homogeneity among the four correlations in this set could not be rejected ($\chi^2 = 2.06$, $P \geq 0.50$, d.f. = 3); thus, results of this analysis do not support the hypothesis that spatial ability is influenced by a sex-linked major gene. Nor is sex linkage indicated by the pattern of correlations for the spatial factor scores. In fact, the highest correlation for AEA families is between mother and daughter and that for AJA families is between father and son. These results suggest that alternatives to the sex-linked, major-gene hypothesis (for example, the sex-modified threshold model¹⁷) for the inheritance of spatial ability should also be considered.

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Relative role of environmental and genetic factors in respiratory adaptation to high altitude

A VARIETY of adaptive mechanisms which aid in the physiological adjustment to the hypoxic environment of altitude have been reported in man¹. Although visitors to high altitudes increase ventilation in response to environmental hypoxia, natives in the Andes and Himalayas show a blunted or absent ventilatory response to acute hypoxia both at rest and during exercise²⁻⁵. Adult high altitude natives in the Andes have larger lungs with a greater gas exchange surface, as judged by physiological tests of lung volume and diffusing capacity, than sea level natives or short term visitors at altitude⁶⁻⁹. Whether these respiratory adaptations in man are determined solely by environment or relate to genetic traits is uncertain. The age at which these pulmonary adaptations to hypoxia occur is not known, neither is it certain whether migrants to and from high altitude reversibly acquire these physiological changes. To investigate further the mechanism of pulmonary adaptations to chronic hypoxia and to clarify the position of genetic factors in this process, we undertook an expedition to Peru from April to August 1975. Control of ventilation and lung volumes were studied in different age groups, from neonatal period to adulthood, both at high altitude and sea level. It was found that the blunted ventilatory response to

hypoxia and large lung volumes developed gradually after birth over a period of many years, and that the phenomena were at least partly reversible at sea level. The results showed that the adaptive response in respiratory physiology were determined by environmental rather than genetic factors.

The high altitude station was in Puno, a town on Lake Titicaca in the Altiplano (3,850 m; barometric pressure = 484 torr; P_{IO_2} = 91 torr) and the laboratory at low altitude was established in Tacna (800 m; barometric pressure = 715 torr; P_{IO_2} = 139 torr). Subjects were selected from the indigenous population of the two areas and from individuals who had migrated to or from high altitude. They were categorised by genetic background, birth place and parents' birth place, and by length of residence at high altitude. Most of the indigenous population of high altitude and migrants from high altitude were Peruvian Indians and those from sea level were mestizos (mixed extraction). Subjects ranged in age from several hours after birth to 50 yr.

The following measurements were made and recorded continuously using a Honeywell ultraviolet polygraph: inspired ventilation by a Fleisch pneumotachograph with integrating circuit; arterial O_2 saturation by Waters Oximeter; end-tidal P_{O_2} with a Beckman O_2 analyser; and P_{CO_2} with Godart capnograph. Neonates and infants were tested using a tight-fitting face mask with minimal dead space while the subjects were asleep in a thermoneutral condition. Children and adults were tested using a mouthpiece and nose clip while they were awake and seated in a chair. Ventilatory sensitivity to hypoxia was measured by both transient and quasi-steady-state techniques¹⁰. The sensitivity was expressed as the ratio between percentage increase in ventilation and percentage decrease in arterial O_2 saturation. A ratio < 1 indicated blunted hypoxic sensitivity; a ratio of up to 5 indicated intermediate response and > 5 a normal response. Ventilatory response to hypercapnia was studied by the conventional rebreathing technique. Lung volumes were measured by recording the largest of three vital capacity manoeuvres in children and adults, and crying vital capacity was measured in neonates and infants¹¹.

In 20 full-term highlander neonates < 5 d old, raising the inspired oxygen concentration from ambient (20.9%) to > 60% produced two types of response; an immediate response which was a small decrease in ventilation, followed by a sustained increase during prolonged oxygen breathing. Lowering the inspired oxygen concentration to 12–15% caused a small transient increase in ventilation and then a depression, which was promptly reversed by raising P_{IO_2} . The pattern of responses to alterations in inspired oxygen was similar in 35 full-term neonates studied at a low altitude. All the subjects, however, showed increased ventilation during rebreathing CO_2 in O_2 . In infants up to the age of 6 months studied at both high and low altitude, lowering the inspired oxygen concentration produced a sustained increase in minute ventilation which promptly decreased on increasing P_{IO_2} . Thus, hypoxic ventilatory drive matures after birth at high altitude as it does at sea level¹².

Except for one 6-yr-old girl and a boy all 43 children up to the age of 12 yr showed an increase in ventilation with acute hypoxia and a decrease with hyperoxia at high altitude. These responses were similar to those of children studied at low altitude, excluding the two exceptions.

Of the 58 high altitude natives between 13 and 20 yr, ~ 30 showed a normal response, 16 an intermediate response, and 10 a blunted response to hypoxia. Acute hyperoxia depressed ventilation in those subjects who responded well to hypoxia. All 40 adult high altitude natives over the age of 22 demonstrated a blunted ventilatory response to hypoxia. Blunted responses occurred independent of the individual's parentage and genetic background. These results showed that a normal hypoxic ventilatory drive was developed in the natives of high altitude before it was substantially lost during adult life. The hypercapnic response in the adults was near normal.

Table 1 Respiratory adaptation to high altitude

Duration of hypoxia	Ventilatory response to acute hypoxia	Increased vital capacity from predicted sea level values
Birth to 1 week	±	0
2–6 months	+++	0
5–8 yr	++++	+
8–12 yr	+++	++
12–20 yr	++	+++
20–25 yr	+	+++

None of the migrants to high altitude demonstrated a diminished ventilatory response to hypoxia, although the longest period of residence at high altitude in migrants was 6 yr. All but one of the 28 migrants from high altitude to sea level who were less than 12 yr old showed normal ventilatory response to acute hypoxia. Recent migrants more than 15 yr old showed a diminished ventilatory response to hypoxia. These results conform with the findings at high altitude. Adult migrants who had been at sea level for long periods, however, usually showed a normal response to hypoxia, suggesting that the acquired blunted hypoxic response was a reversible phenomenon. Loss and reversal occurred over a prolonged period.

Vital capacity expressed in absolute terms or as ml per kg body weight was similar in 12 high altitude native neonates and in 20 sea level neonates, and was generally within the range of normal predicted values for crying vital capacity data¹¹. Crying vital capacity tended to be similar in low and high altitude infants less than 1 yr old. High altitude children between 9 and 13 yr old had vital capacities that averaged 2.87 ± 0.62 l, or $121 \pm 13.7\%$ of predicted values based on age and height. The accelerated lung growth curve persisted throughout the teenage years, so that native young adults 18–21 yr old had vital capacities which were 5.05 ± 0.67 l, or $145 \pm 16.7\%$ of predicted, whereas vital capacity of sea level controls was 3.73 ± 0.31 l, or $104 \pm 8.2\%$ of predicted. The increase above predicted values of vital capacity in highlanders occurred independently of genetic background and place of birth of the parents. Thus, the normal vital capacity that occurs with age was greater at high altitude than at sea level.

In general, individuals of any age who had migrated to high altitude tended to have normal or near normal vital capacities if their stay at altitude was < 3 yr. Those migrants (ages 7, 9, 10 and 18 yr) who had lived at high altitude for more than 4 yr had vital capacities considerably above predicted values.

Thus the ventilatory response to hypoxia and vital capacity are similar in neonates and infants at sea level and at high altitude. Vital capacity tends to increase in the postnatal period and continues to do so throughout the teenage period. The blunted hypoxic response, however, only begins to manifest itself in teenage life, becoming universal in adults.

Our results, which are summarised in Table 1, suggest that the characteristic diminished ventilatory response to hypoxia and large lung volumes found in adult high altitude natives are determined environmentally, rather than genetically. A genetic trait tends to be excluded on the grounds that offspring of lowlanders born and bred at high altitude showed the same phenomena as native highlanders. A similar developmental adaptation of improved physical performance capacity in response to hypoxia has been reported¹³.

The similarity in the lack of a sustained hypoxic ventilatory drive in sleeping neonates at sea level and high altitude suggests that the normal hypoxic control of ventilation develops fully only after birth. A reflex hypoxic drive is thus of limited value for survival in newborns at high altitude. In addition, newborns at low and high altitude have similar ventilatory control mechanisms and lung volumes, suggesting that no genetic or intrauterine adaptive changes occur in these parameters in

preparation for life at high altitude. Appropriately, however, an increase in placental surface area relative to the body weight of the foetus occurs at high altitude¹⁴. It is presumably only after birth that high altitude neonates are exposed to a greater hypoxic stimuli than at sea level. The adult pattern of responses and adaptations to hypoxia begins to develop shortly after birth. In teenage life a variety of effective gas transport adaptations develop, such as the increased area for gas exchange provided by large lungs, and ventilatory hypoxic drive assumes a less important role.

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Elimination of aziridine residues from chemosterilised mosquitoes

CRITICISM^{1–4} of the use of the alkylating agent thiotepa (triethylene thiophosphoramidate) to sterilise mosquitoes, as part of an eradication programme in India, focused on its toxicity and the possibility that it or its breakdown products would harm other animal components of the food chain. For example, a Canadian team found reduced fertility in spiders fed on mosquitoes sterilised with thiotepa⁵. Some means was needed to ensure that no aziridine residues—breakdown products of thiotepa—remain in the insects after release into the environment. We have now achieved this, essentially by dipping the chemosterilised insects into acid and then alkaline solutions.

Complete breakdown of 3.1% thiotepa solution has been reported to occur in 90 min at pH 4, but aziridine activity persists for 24 h. This is due to the production of ethyleneimine which is stable in acid medium and tends to accumulate. Ethyleneimine is cytotoxic and mutagenic and may in some cases be carcinogenic⁶ and therefore potentially

hazardous. It breaks down to non-aziridine compounds at pH 9 (ref. 7).

The decomposition of thiotepa in acid medium was monitored by bioassay. A 0.6% solution was prepared in 0.01 N H₂SO₄, pH 1.8–2.0, and pH 3 was maintained by addition of small increments of N H₂SO₄ for 90 min. A hundred mosquito pupae (*Culex pipiens fatigans*) were placed in this solution for 3 h, washed twice in tap water and held in cages at 28–29 °C and 75% relative humidity until emergence. An equal number of virgin females was introduced into the cages and egg rafts were collected for routine bioassay. Fifty rafts, scored for percentage hatch, gave 92.7% fertility, whereas the controls dipped in water and in 0.6% thiotepa (pH 8) showed 94.9% and 0.01% fertility, respectively. Breakdown of thiotepa was complete in acid solution and the main decomposition product, ethyleneimine, proved inactive as a sterilant for *C. p. fatigans*. Therefore dipping sterilised pupae in acid solution (0.0025 N H₂SO₄, pH 2.6–2.8) for 90 min would ensure complete decomposition of thiotepa to ethyleneimine. Although no appreciable accumulation of ethyleneimine is likely, traces present as the intermediate decomposition product in sterile males may contaminate the environment. Ethyleneimine is hydrolysed to deuteriophosphoric acid and D₂NCH₂-CH₂OD, and this conversion can be accelerated by dipping acidified pupae in alkaline solution, pH 9 (ref. 7), thus making the mosquitoes free of any traces of aziridine compounds.

We tested the biological fitness of sterile male *C. p. fatigans* produced when pupae were dipped in 0.6% thiotepa, pH 8, for 3 h, washed twice in tap water, dipped in 0.0025 N H₂SO₄ for 90 min, washed in tap water, dipped in alkaline solution, pH 9.1–9.4, for 90 min, washed and transferred to tap water for emergence. No pupae died after this treatment. To assess survival, 100 sterile and 100 normal males were held in cages (ten replicates) and offered water-soaked raisins daily. The number of mosquitoes found dead in each cage was recorded at 24-h intervals. Weekly (%) mortality of sterile males averaged 1.9, 12.7 and 17.4 (cumulative total 32.2 in 3 weeks) and controls averaged 3.6, 13.2 and 20.3 (cumulative total 37.1 in 3 weeks). Thus the treatment had no effect on the longevity of the mosquitoes.

Sterile males were also tested for mating competitiveness. In ten laboratory cages, 100 sterile and 100 normal, 1–2-d-old males and 100 virgin, 2-d-old females were fed on raisins and allowed to mate for 4 d. They were offered pigeons for a blood meal. A total of 622 egg rafts was collected, of which 327 were sterile. As sterility averaged 52.57%, sterile males proved competitive.

Thus residues of thiotepa, and other aziridine compounds can be eliminated from sterile mosquitoes by dipping them in acid and alkaline solutions. Residue-free emergent adults survive well for at least 3 weeks. As sterile males compete favourably with normal males in the insemination of females, mass release of these sterile males for population control would not be hazardous to the food chain and would not contaminate the environment directly or indirectly.

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Augmentation of human monocyte chemotactic response by levamisole

THE antihelminthic drug, levamisole ((-)-tetramisole) has been shown to possess potent immunostimulatory properties as demonstrated by its ability to enhance delayed hypersensitivity responses in aged individuals and in patients with cancer^{1,2}. In addition, animals treated with (-)-tetramisole have heightened activity of their reticuloendothelial systems as measured by clearance of carbon particles and phagocytosis of sensitised sheep erythrocytes^{3,4}. Although the mechanism of action of levamisole remains obscure, its pharmacological properties suggest that it may affect macrophage function and as such could be a useful tool to probe the pathophysiology of this cell. This report describes the effect of (-)-tetramisole and its isomers on monocyte chemotaxis *in vitro*, and demonstrates a potential mechanism of action for these drugs as well as their usefulness for delineating mechanisms of leukocyte chemotactic responsiveness.

Human monocyte chemotaxis was assayed *in vitro* by a minor modification of a previously described technique⁵. Briefly, peripheral blood monocytes were isolated on Ficoll-Hypaque gradients, washed extensively, and resuspended to contain 1.5×10^6 monocytes ml^{-1} in medium RPMI 1640 (Grand Island Biological Company, Grand Island, New York). Cell suspension (0.4 ml) was placed in the upper compartment of a modified Boyden chamber and separated from the chemotactic or control stimulant in the lower compartment by a 5.0- μm polycarbonate (Nuclepore) filter. After incubation for various times at 37 °C in humidified air, the chambers were emptied, the filters removed, fixed in ethanol and stained with haematoxylin. Chemotactic response was assessed by counting and averaging the number of cells which migrated completely through the filter per microscopic field in 20 oil immersion fields ($\times 1,540$). All assays were carried out in triplicate, each experiment repeated at least twice with cells from different donors and a single representative experiment presented.

Human polymorphonuclear leukocyte (PMN) chemotaxis was quantified similarly⁶. PMNs were isolated from whole blood by sedimentation with 3% Dextran in saline, washed extensively, and resuspended to 2.2×10^6 PMN ml^{-1} in Gey's balanced salt solution (BSS) containing 2% bovalbumin (Flow Laboratories, Rockville, Maryland), pH 7.2. A 5.0- μm nitrocellulose filter (Millipore) was used to separate the upper and lower compartments of the chemotaxis chambers. After incubation for 3 h at 37 °C, the filters were processed as described previously⁶. Chemotactic response was expressed as the average number of PMNs migrating completely through the filter per field in 20 high power fields ($\times 780$).

To determine the effect of (-)-tetramisole, its stereoisomer, (+)-tetramisole, and *p*-Br(-)-tetramisole (Janssen, Beerse, Belgium) on monocyte chemotaxis, the isolated cells were incubated with various doses of the drugs contained in RPMI 1640 or with medium alone, and tested for chemotactic response to lymphocyte-derived chemotactic factor (LDCF), endotoxin-activated human serum (AHS) or to the chemotactically active peptide, *N*-formyl-methionyl-phenylalanine (f-met-phe)⁷. Table 1 illustrates that doses of (-)-tetramisole ranging from 10^{-5} – 10^{-3} M enhanced the chemotactic response of monocytes to the three chemotactic factors by 20–148%. Similar doses of (+)-tetramisole had no significant ($P > 0.2$) effect on monocyte chemotactic responsiveness to LDCF. Cells incubated with *p*-Br(-)-tetramisole (10^{-6} – 5×10^{-5} M) also exhibited an increased chemotactic response to LDCF; however at doses higher than 5×10^{-4} M, *p*-Br(-)-tetramisole depressed chemotaxis and was toxic to the monocytes as judged by Trypan blue ex-

clusion. The effect of (-)-tetramisole was removed by washing the cells extensively after a 30-min incubation with the drug. Enhanced monocyte chemotactic responsiveness was not seen when (-)-tetramisole was placed in the lower compartment of the chemotaxis chamber and incubated with the chemotactic factors rather than with the cells. These data indicated that (-)-tetramisole exerts its effect primarily on the monocyte, and that enhancement of monocyte chemotactic responsiveness by (-)-tetramisole is stereospecific.

To determine whether enhancement of monocyte chemotaxis by (-)-tetramisole required binding of the drug to the monocytes, cells were incubated with medium containing various doses of (-)-tetramisole alone or with medium containing (-)-tetramisole plus (+)-tetramisole, and tested for chemotactic response to LDCF. Figure 1 illustrates that doses of 10^{-4} and 10^{-3} M (+)-tetramisole reduced the enhancing effect of (-)-tetramisole. The parallel nature of the curves indicated that (+)-tetramisole inhibited the enhancing activity produced by (-)-tetramisole by competitive binding (k_D for (-)-tetramisole = 1×10^{-4} ; k_D for (+)-tetramisole = 0.8×10^{-4}). To ascertain whether (-)-tetramisole increased the rate of cell migration or the total number of cells capable of responding to a given dose of chemotactic factor, monocytes were incubated (37 °C for 30 min) with RPMI containing 10^{-3} M (-)-tetramisole or with medium alone, and the number of cells responding to LDCF determined after various times of incubation in the chemotaxis chambers. Figure 2 illustrates that (-)-tetramisole increased both the rate of monocyte chemotactic responsiveness and the maximum number of cells responding chemotactically.

We next determined whether (-)-tetramisole exerted an effect on another type of cell capable of responding chemotactically. Human PMNs were incubated with Gey's BSS

Table 1 Effect of isomers of tetramisole on human monocyte chemotactic response

Monocytes incubated with*	Chemotactic activity† of monocytes in response to:		
	LDCF‡	AHS	f-met-phe¶
Medium alone	43.9 ± 3.6	79.5 ± 6.3	36.0 ± 3.0
(-)-Tetramisole			
1×10^{-3} M	98.0 ± 1.0	114.8 ± 7.3	89.4 ± 1.7
1×10^{-4} M	73.9 ± 6.9	107.6 ± 10.2	53.1 ± 4.3
1×10^{-5} M	59.1 ± 0.3	95.4 ± 1.3	44.9 ± 5.3
<i>p</i> -Br(-)-tetramisole			
5×10^{-5} M	82.5 ± 6.8	—	—
1×10^{-6} M	61.7 ± 3.5	—	—
1×10^{-6} M	49.8 ± 2.1	—	—
(+)-Tetramisole			
1×10^{-3} M	49.8 ± 5.3	—	—
1×10^{-4} M	49.2 ± 2.0	—	—
1×10^{-5} M	45.5 ± 5.6	—	—

*Human monocytes were isolated on Ficoll-Hypaque gradients, washed extensively, and resuspended ($1.5 \times 10^6 \text{ ml}^{-1}$) in medium containing various molar concentrations of the indicated isomers of tetramisole or in medium alone. After incubation (37 °C for 30 min) the monocytes were placed in the upper compartment of a modified Boyden chamber and tested for chemotactic response.

†Chemotactic activity is expressed as the average number of monocytes in triplicate samples migrating completely through a 5.0 μm polycarbonate filter per oil immersion field ($\times 1,540$) $\pm$ s.e.m.

‡Lymphocyte derived chemotactic factor (LDCF) was obtained from supernatants of concanavalin A-stimulated lymphocyte cultures. LDCF was diluted to 15% v/v in RPMI 1640 for use as a chemotactic stimulant.

||Activated human serum (AHS) was prepared by incubation of normal serum with *S. typhosa* endotoxin (1 mg ml^{-1} serum) for 60 min at 37 °C followed by 56 °C for 30 min. AHS was then diluted to 0.5% v/v in RPMI 1640 for use as a chemotactic stimulant.

¶f-met-phe was diluted to 10^{-6} M ml^{-1} RPMI 1640 for use as a chemotactic stimulant.

containing 10^{-3} – 10^{-6} M (–)-tetramisole or with medium alone and tested for chemotactic responsiveness to various doses of AHS or to f-met-phe. At the doses tested, (–)-tetramisole had no effect on PMN chemotactic response to the two chemotactic factors, indicating a differential effectiveness of the drug for monocytes.

The above results demonstrate that (–)-tetramisole and p-Br-(–)-tetramisole exert stimulatory effects on human monocyte chemotactic response by increasing both the rate of cell migration and the total number of cells capable of responding to a given dose of chemotactic factor. In addition, it seems that the enhancing effect is stereospecific and that the drug must bind to the monocytes since addition of the dextroisomer competitively inhibited the enhancing effect of (–)-tetramisole. Both isomers have approximately the same dissociation constant for monocyte binding ($k_D \sim 10^{-4}$).

Our data demonstrate that (–)-tetramisole stimulates the unidirectional migration of monocytes in response to a given number of chemotactically active molecules. The ability of (–)-tetramisole to enhance monocyte chemotactic function might be one mechanism by which it augments immunological response *in vivo*. The mechanism of action of (–)-tetramisole on human monocyte chemotaxis has not yet been determined but it could affect binding of chemotactic factors to the monocyte or the translation of the chemotactic factor–monocyte interaction into a directional cell movement. Little information concerning the mechanism by which chemotactic factors induce unidirectional leukocyte migration is available. The stereospecificity as well as cell-type specificity of the effect of (–)-tetramisole indicate that this drug has differential cell membrane effects on chemotactically responsive cells. This drug and its isomers can therefore be useful tools in delineating mechanisms of unidirectional cell migration.

Fig. 1 Competitive inhibition of the enhancing effect of (–)-tetramisole on monocyte chemotactic response to (+)-tetramisole. Isolated monocytes were incubated (37°C for 30 min) with medium containing 10^{-3} – 10^{-6} M (–)-tetramisole alone (●), or with 10^{-3} – 10^{-6} M (–)-tetramisole plus 10^{-3} M (▲) or 10^{-4} M (+)-tetramisole (■), and tested for chemotactic responsiveness to LDCF (15% v/v). k_D for (–)-tetramisole = 10^{-4} ; k_D for (+)-tetramisole = 0.8×10^{-4} . % Enhancement =

$$\left(\frac{\text{Chemotactic responsiveness of cells incubated with drugs}}{\text{Chemotactic responsiveness of cells incubated with medium alone}} - 1 \right) \times 100.$$

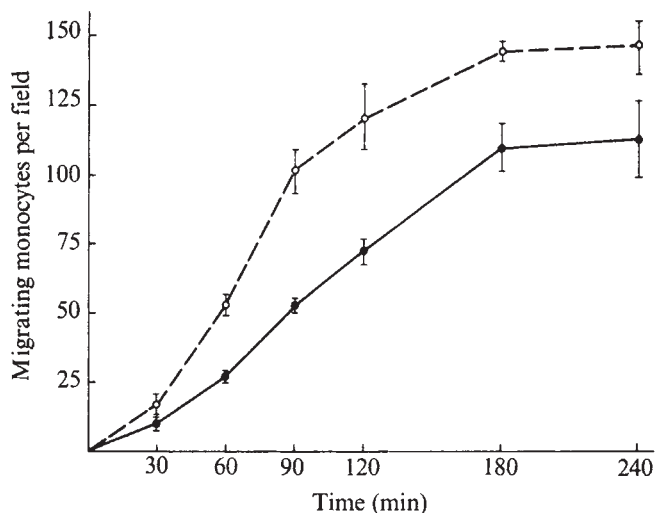
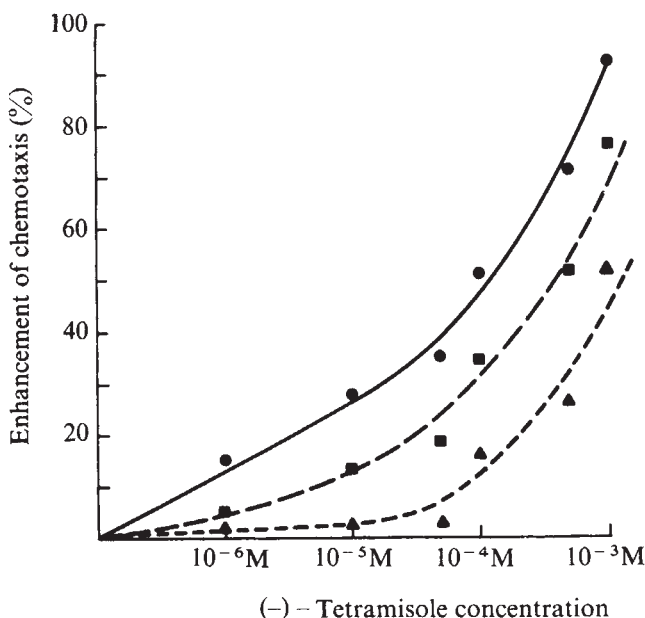


Fig. 2 Kinetics of enhancement of monocyte chemotactic response to (–)-tetramisole. Isolated monocytes were incubated (37°C for 30 min) with medium alone (●) or containing 10^{-3} M (–)-tetramisole (○), and tested for chemotactic response to LDCF (15% v/v) after the indicated incubation time in the chemotaxis chamber. Vertical bars indicate s.e.m. for triplicate samples.

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A new differentiation antigen defining a subpopulation of mouse T cells

It is generally accepted that lymphocyte reactions such as the mitogenic response to concanavalin A (con A) and phytohaemagglutinin (PHA), the graft *versus* host (GvH) reaction, the helper effect in the antibody response and cell-mediated cytotoxicity (CMC) all involve the activity of thymus-derived lymphocytes (T cells). Because of the paucity of techniques for identifying cells with different functions, however, very little is known about the identity and relationships of T cells involved in these reactions.

In the mouse, a convenient way of identifying functionally distinct subpopulations of T cells has been described by the demonstration that Ly antigens can be used to distinguish between such subpopulations^{1,2}. Here we describe an antigenic marker that enables the distinction between cytotoxic ('killer') T cells from T cells involved in the above reactions. Following examples of other previously described differentiation antigens demonstrated by heteroantisera (that is, MBLA³, MSPCA⁴ and MSLA⁵) we have tentatively

Table 1 Suppression of CMC function of spleen cells (SPC) and non-adherent peritoneal cells (NAPC) from H-2-primed donors by pretreatment with Ra-nu/nu and Ly 3.2 antisera and complement (C)

Cells*	Pretreatment†	c.p.m.	CMC‡ %	% Reduction
Non-immune SPC	Diluent only	2,657	0	—
Immune SPC	Diluent only	288	89	—
Immune SPC	Normal rabbit serum + C	354	87	Standard
Immune SPC	Ra-nu/nu + C	2,447	8	91
Non-immune NAPC	Diluent only	8,565	0	—
Immune NAPC	Diluent only	1,072	87	—
Immune NAPC	Normal rabbit serum + C	1,178	86	Standard
Immune NAPC	Ra-nu/nu + C	6,992	18	80
Immune NAPC	Normal mouse serum + C	1,255	85	Standard
Immune NAPC	Anti Ly 3.2 + C	7,043	18	80
(Immune NAPC Ra-nu/nu + C) + (Immune NAPC anti-Ly 3.2 + C)		6,900	19	77

*B6 donors received a single subcutaneous injection of 2×10^7 BALB/c Meth A sarcoma cells (immune SPC) or multiple intraperitoneal injections of 5×10^6 BALB/c (immune NAPC) or B6 syngeneic (non-immune) spleen cells. SPC were collected 10 d after an injection and NAPC 3 d after the last injection of four consecutive weekly injections.

†For pretreatment, equal volumes of cells (10^7 ml⁻¹), antiserum (Ra-nu/nu diluted 1:2, anti-Ly 3.2 diluted 1:15) and complement (C; guinea pig serum at 1:3 with Ra-nu/nu antiserum and rabbit serum at 1:15 with Ly 3.2 antiserum) were incubated in 5% CO₂ in air for 45 min at 37 °C. The cells were then washed twice and resuspended in culture medium with or without adjustments to the same concentration of viable cells: in all cases similar results were obtained. Earle's balanced salt solution with 2.5% foetal bovine serum and 5×10^{-5} M 2-mercaptoethanol was used for cell preparation and as diluent for antisera and C.

‡³H-proline CMC microassay²¹, in which the radioactivity of surviving attached monolayer target cells is measured. Washed SPC or NAPC (pretreated as described above; 10^5 per well) were added to a ³H-proline labelled monolayer of BALB/c sarcoma cells (10^3 per well) for 12 h. CMC percentage and percentage reduction of CMC were calculated as described previously¹⁰.

called the antigenic determinant(s) present on cytotoxic T cells, mouse killer T-cell antigen (MKTCA).

The antiserum was prepared by intravenous immunisation of a New Zealand rabbit with spleen cells of nu/nu mice, known to be deficient in thymus and T cells⁶ but not in T-cell precursors^{7,8}. Male nu/nu mice that served as donors of SPC were derived from a stock that has undergone 4–5 backcrosses to BALB/c mice. The inoculation of 1.7×10^8 nu/nu SPC was followed 7 d later by additional inoculation of 7.5×10^8 cells. The rabbit was bled 1 week after the second inoculation. The serum was heat inactivated for 30 min at 56 °C, diluted 1:4 with medium 199 and absorbed repeatedly *in vitro* for 30 min on ice with 10–20% (v/v) of packed cells. After one absorption with erythrocytes and one with thymic leukaemia EL4, six consecutive absorptions with thymocytes of different strains were carried out to remove activity against thymocytes as assessed in complement (C)-dependent cytotoxicity assay⁹. Absorbed antiserum, now called Ra-nu/nu was divided into small aliquots and kept at -70 °C until used.

Our earlier work⁹ established that Ra-nu/nu antiserum possesses two different specificities. In addition to activity against bone marrow-derived lymphocytes (B cells) as demonstrated by inhibition of IgM plaque-forming cells and by cytotoxicity against myelomas, the antiserum has strong activity against cytotoxic T cells⁹. Activity against B cells can be absorbed with foetal liver cells but not with lymph node cells⁹ and activity against cytotoxic T cells can be absorbed with lymph node cells but not with foetal liver cells and thymocytes (see below).

Results of typical experiments demonstrating strong inhibition of CMC by pretreatment with Ra-nu/nu antiserum and C of alloimmune spleen cells and non-adherent peritoneal lymphocytes are shown in Table 1. T-cell dependence of CMC is indicated by the strong suppression of the reaction by pretreatment with anti-Ly 3 serum (specificity anti-Ly 3.2), which is specific for alloimmune cytotoxic T cells^{10,11}. We have shown previously¹⁰ that inhibition of CMC in our system is due to the elimination of T cells with antibody and C, and not to the nonspecific blocking by antigen-antibody complexes which may be operative in systems measuring non-T cell killing¹². Moreover, the elimination of B cells by pretreatment with various anti-B-cell specific sera and C, had no effect on CMC (H. S., unpublished). It might be argued, however, that cooperation of other cells is necessary for CMC and that inhibition of the

reaction by Ra-nu/nu antiserum and C is due to elimination of the accessory non-T cells. The failure to reconstitute CMC by mixing cells pretreated with anti-Ly 3 serum and C with cells pretreated with Ra-nu/nu antiserum and C (compare line 11 with lines 8 and 10) rules out this possibility and indicates that cells sensitive to both antisera belong to the same subpopulation of T cells. The evidence that Ly 3 and MKTCA antigens are not the same comes from absorption experiments reported below.

In contrast to killer T cells, those carrying out the other functions tested remained unaffected by pretreatment with Ra-nu/nu antiserum and C. Table 2 shows results demonstrating the lack of inhibition of helper effect. This lack of inhibition was similarly observed in the case of the mitogenic response of spleen cells to PHA and con A and in the case of the GvH reaction of parental spleen cells injected into newborn F₁ hybrids (data not shown). Each function was tested in two or more independent experiments; in all cases similar results were obtained. These results indicate that killer cells differ in their surface antigenic properties from T cells cooperating with B cells in the antibody response, from cells responsible for GvH reactions and from cells responding to PHA and con A. This confirms and extends the evidence^{11,13–16} that cells

Table 2 Helper effect: lack of inhibition by Ra-nu/nu antiserum and C

No. of primed cells per culture	Pretreatment of primed cells†	No. of TNP-PFC per culture on day 4* Expt a	Expt b
0	—	750	150
1×10^6	Diluent only	4,000	3,400
2×10^6		7,250	5,900
1×10^6	Normal rabbit serum + C	3,600	2,000
2×10^6		5,250	4,500
1×10^6	Ra-nu/nu + C	5,250	2,050
2×10^6		6,000	4,550

*Three B6 mice were injected intravenously with 0.1 ml 0.01% (v/v) sheep red blood cells (SRBC). After 4 d their spleens were pooled and pretreated with sera and C as described. Mishell-Dutton-type cultures were prepared containing increasing numbers of these pretreated primed cells (as indicated) with unprimed cells maintaining constant total 10^7 cells per culture. The cultures were sensitised with trinitrophenyl (TNP)-conjugated SRBC and assayed 4 d later for TNP-specific plaque-forming cells (TNP-PFC)²².

†See Table 1.

causing CMC belong to a distinct subpopulation of functional T cells.

To determine the tissue distribution of MKTCA, absorption experiments were carried out. Results are summarised in Table 3. They show that MKTCA is present on cells from the lymph nodes and bone marrow of normal mice, as well as on spleen cells from *nu/nu* mice. It is absent, however, from normal foetal liver cells and thymocytes. This pattern of tissue distribution is different from that of Ly 3 alloantigen¹⁷, indicating that although both antigens are characteristic of alloimmune killer cells, they are not the same. The relationship of MKTCA and Ly 3 antigens to Ka—another heteroantigen present on alloimmune killer cells¹⁸—is not known, as no other functional T cells have been examined for the presence of this antigen.

Table 3 Summary of absorption experiments with Ra-*nu/nu* antiserum showing the distribution of MKTCA antigen in lymphoid and haemopoietic tissues

Cells from	No. of cells used for absorption*	% Reduction in CMC†	Result of absorption
Thymus	5 × 10 ⁸	72	—
Lymph nodes	5 × 10 ⁸	9	+
Bone marrow	2.5 × 10 ⁸	—2	+
<i>nu/nu</i> spleen	3.5 × 10 ⁸	—16	+
Foetal liver	3.5 × 10 ⁸	88	—

*Ra-*nu/nu* antiserum (0.5 ml) was absorbed with indicated number of cells for 30 min on ice. After absorption serum was diluted 1:2 and tested against non-adherent peritoneal cells (NAPC) from H-2-primed mice as described in Table 1.

†Mean value calculated from two to three experiments.

It is known that bone marrow from normal adult mice as well as spleen cells from *nu/nu* mice contain precursors of T cells^{7,8,19}. Thus the ability of cells from these tissues to absorb anti-MKTCA activity suggest that the antigen is also present on the prethymic precursors of killer T cells.

Differentiation of T cells is generally depicted as involving transformation of prethymic cells to immunoincompetent thymocytes, which themselves are precursors of functionally mature, immunocompetent T cells²⁰. The absence of MKTCA from thymocytes thus raises several possibilities which may have implications for the understanding of differentiation pathways of killer T cells. MKTCA should therefore prove to be a useful marker for further delineation and clarification of the differentiation stages involved in the process of generation of functionally distinct subpopulations of T cells. Although the majority of functional assays were carried out with cells of B6 mice, preliminary experiments in BALB/c mice and the fact that the antiserum has been produced in a heterologous system suggest that MKTCA is also characteristic of killer T cells of other strains.

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Virus-specific T-cell-mediated cytotoxicity across the H-2 barrier to virus-altered alloantigen

ALTHOUGH the biological function of the structures coded in the major murine histocompatibility (H-2) gene complex is poorly understood, some of these structures are crucially involved in most, if not all, cell-mediated immune functions^{1,2}. To express their immunological function, thymus-derived lymphocytes (T cells) from conventional mice and the cells with which they interact must share part of the H-2 gene complex. T helper cells and bone marrow-derived lymphocytes (B cells) usually must be compatible for the *I* region of H-2 (ref. 3) to act cooperatively in T-cell dependent antibody responses. This region codes for Ia cell surface antigens and immune response (*Ir*) genes. Also, delayed type hypersensitivity to fowl γ -globulin can be transferred adoptively to intact mice only within *I*-region compatible donor-recipient combinations⁴. Distinct from these models, virus-immune T cells lyse virus-infected target cells *in vitro* only when both are compatible at the *K* and/or *D* regions of H-2 (refs 5–7). *I*-region compatibility is neither necessary nor sufficient on its own. Also T-cell-mediated lysis of chemically modified targets⁸ and cytotoxicity against cells expressing either the minor histocompatibility antigens⁹ or the male Y antigen¹⁰ are similarly restricted.

Two probably mutually exclusive hypotheses have been proposed to explain this apparent restriction^{5,6}. First, according to the 'physiological interaction' hypothesis, T cells specifically recognise antigens on the target cell surface; however, for cooperation or lysis to occur, self to self-like interactions are necessary. The structures responsible for such physiological interactions would have to be coded in the *I* region of H-2 for T helper cell functions³, but in *K* and *D* for cytotoxic interactions^{5,6}. Second, the altered self hypothesis proposes that T cells interact with other cells exclusively by way of the immunologically specific T-cell receptor. For cytolytic interactions this receptor is specific for virally, or for chemically or genetically (alloantigens), altered self structures coded in *K* and *D* regions (refs 5 and 6). On T helper cells this receptor may be specific for antigen-altered structures which are coded in the *I* region of H-2.

Most of the experimental evidence—at least that derived from competitive inhibition experiments *in vitro*⁵ using the murine lymphocytic choriomeningitis virus (LCMV) model, selective proliferation studies *in vivo*⁵ and experiments with H-2 mutant mice^{1,12}—is explained best by the altered self idea. Also, cytotoxic activity against alloantigens (being a form of altered self) could be accommodated readily within this concept^{5,6,12}. A physiological interaction hypothesis, however, is a possible explanation if the expression of physiological interaction structures is controlled by very complex genetic regulatory mechanisms^{11,12}.

If virus-specific cytotoxic T cells are specific for altered self,

Table 1 Virus-specific cytotoxic activity in spleens of LCMV-infected P→F₁ bone marrow chimerae*

Spleen cells from LCMV-infected mice† (H-2 type)	Cell treatment (% dead cells after treatment)	% ⁵¹ Cr release from target cells‡					
		L929 (H-2 ^k)		J774 (H-2 ^d)		Uninfected	
		LCMV infected 10:1	3:1	LCMV infected 10:1	3:1	LCMV infected 10:1	Uninfected 10:1
C3H→C3D2 F ₁ (H-2 ^k) (H-2 ^k × H-2 ^d)	None (7)	67	51	24	98	73	41
	Anti-H-2 ^k +C' (> 98)	27	26	26	35	38	40
	Anti-H-2 ^d +C' (12)	68	50	26	85	66	40
	Normal B10.D2+C' (13)	64	48	25	86	66	40
C3H (H-2 ^k)	None (5)	59	42	29	43	40	41
	Anti-H-2 ^k +C' (> 98)	25	24	23	—	ND	—
	Anti-H-2 ^d +C' (10)	58	40	24	—	ND	—
	Normal B10.D2+C' (12)	56	39	24	—	ND	—
C3H Normal	None	24	ND	24	40	ND	40
BALB/c (H-2 ^d)	None (12)	28	25	25	88	72	42
	Anti-H-2 ^k +C' (15)	—	ND	—	85	63	41
	Anti-H-2 ^d +C' (> 98)	—	ND	—	39	38	40
	Normal B10.BR+C' (16)	—	ND	—	84	64	39
BALB/c Normal	None	27	ND	25	38	ND	30

*Mice were obtained from Jackson Laboratories, Bar Harbor, Maine and used at 7–10 weeks of age. (C3H × DBA/2) F₁ were irradiated with 900 r and reconstituted with 2 × 10⁷ anti-θ+C'-treated C3H bone marrow cells. Chimerae were infected intravenously with 10⁸ intracerebral LD₅₀ of the WE strain of LCMV 11 weeks later. 7-d immune spleen cells were used.

†Anti-H-2 sera were obtained by hyperimmunising B10.BR (H-2^k) mice with B10.D2 (H-2^d) spleen cells and vice versa. Both antisera killed > 90% specifically when assayed at a 1:30 dilution and 5 × 10⁶ spleen cells ml⁻¹. A selected rabbit complement was used unabsorbed at a 1:8 final concentration. After the treatment viability was determined by Trypan blue exclusion. The antisera were used at a 1:3 final dilution and 5 × 10⁶ spleen cells ml⁻¹. The anti-H-2 sera were highly specific as indicated by the percentage of dead cells found after treatment. Cells were resuspended according to the original viable cell concentration before treatment (groups none) and assayed. Decrease of activity by nonspecific antiserum+C' was not different from that caused by normal control serum+C'.

‡⁵¹Cr release assay was carried out as described previously^{6,8}; J774 target cells were provided by Dr M. Bevan, Salk Institute, La Jolla, California. Spleen cells were assayed at the spleen cell:target ratios of 10:1 and 3:1 for 12 h at 37 °C; results are means of triplicates; s.e.m. was 0.4–1.5 for L929 cells and 0.7–2.4 for J774 cells. LCMV-immune spleen cells tested on infected targets caused statistically significant ⁵¹Cr release when compared with release from normal targets or with the activity of normal spleen cells.

||P < 0.01.

ND, Not determined.

it should be possible to sensitise T cells specifically to virus-altered alloantigen across the H-2 barrier. This report demonstrates that virus-specific cytotoxic T cells from chimaeric mice can lyse across the H-2 barrier only those infected allogeneic targets to which they are tolerant. Thus, reactivities to virus-specific altered self, to virus-altered alloantigen or to alloantigen itself are obviously comparable^{5,6,12}.

To overcome the strong normal reactivity to alloantigens that could mask potential but experimentally undetectable reactivity for virus-altered alloantigen, alloantigen tolerant P→F₁ hybrid irradiation chimerae reconstituted with bone marrow of one parent were used¹³. LCMV-infected C3H→(C3H × DBA/2) F₁ chimerae generated cytotoxic T cells, with exclusively H-2^k surface characteristics, which lysed

Table 2 T-cell dependence of cytotoxic activity of LCMV-infected P→F₁ bone marrow chimerae*

Spleen cells from LCMV-infected mice* (H-2 type)	Cell treatment* (% dead cells after treatment)	% ⁵¹ Cr release from LCMV-infected target cells†		
		L929 (H-2 ^k)	J774 (H-2 ^d)	C57BL/6‡ (H-2 ^b)
C3H→C3D2 F ₁ (H-2 ^k) (H-2 ^k × H-2 ^d)	None (15)	49	60	35¶
	Anti-H-2 ^k +C' (> 98)	14¶	24¶	ND
	Anti-H-2 ^d +C' (18)	48	57	ND
	Normal AKR+C' (19)	46	58	ND
	AKR anti-θ C3H+C' (54)	15	25¶	ND
C3D2 F ₁ (H-2 ^k × H-2 ^d)	None (14)	54	61	36¶
	Anti-H-2 ^k +C' (> 97)	16¶	24¶	ND
	Anti-H-2 ^d +C' (> 95)	14¶	25¶	ND
	Normal AKR+C' (18)	52	60	ND
	AKR anti-θ C3H+C' (50)	17¶	23¶	ND
C57BL/6 (H-2 ^b)	None (16)	16	26	87
Normal C3H → C3D2 F ₁	—	15	23	37
Normal C3D2 F ₁	—	15	21	38
Normal C57BL/6	—	17	27	35

*Mice, immunisation and antisera, see Table 1. AKR anti-θ C3H was purchased from Bionetics, Kensington, Maryland 20795 (Cat. 8301-01, Lot No. 231-61-5). With a selected rabbit complement C' thymocytes were lysed to 50% at a 1:40 dilution of the anti-θ antiserum. The antiserum was used at a 1:8 final dilution. In these conditions anti-SRBC PFC were not lysed⁵.

†The cytotoxicity assay was carried out as described previously^{5,8}. Spleen cells were assayed at a 30:1 ratio for 6 h at 37 °C. Results are means of triplicates; s.e.m. were 0.3–0.9 for L929, 0.5–1.7 for J774 and 0.9–2.2 for C57BL/6 macrophage targets. LCMV-immune spleen cells caused statistically significant lysis of H-2 compatible or the tolerant H-2 haplotype targets; treatment with irrelevant anti-H-2 antiserum+C' or control sera + C' did not cause loss of activity. Treatment with AKR anti-θ C3H+C' or with the relevant anti-H-2 antiserum abolished all activity; residual activity was not significantly different from normal spleen cells on infected targets.

‡Peritoneal macrophages from normal mice were used as targets as described previously⁶.

||P < 0.01.

¶Not significant.

ND, Not determined.

virus-infected H-2^d target cells but not uninfected H-2^d targets (Table 1). These T cells are tolerant for H-2^d, but can readily react to virus-altered alloantigen. Comparable results were obtained in separate experiments with five C3H→(C3H×DBA/2)F₁, some infected with vaccinia virus instead of LCMV (R.M.Z., unpublished). The cytotoxic effector cells of H-2^k type from chimaeric mice are T cells, as shown by their susceptibility to AKR anti-θ C3H+complement treatment (Table 2). This notion is further supported by the fact that they lysed infected target cells of the tolerant allogeneic parental H-2^d type but were still H-2 restricted in that they could not lyse infected targets of an unrelated allogeneic H-2^b haplotype (Table 2). These results add strong circumstantial evidence in favour of the altered self hypothesis. Our data suggest that altered self, alloantigen, and altered alloantigen are comparable antigens, since all are attacked by T cells because they differ from the normal unaltered cell-surface self marker.

The interaction model has been extended to accommodate similar findings in which T helper cells from chimaeric mice collaborated with H-2I region-incompatible B cells^{13,14}. In this case, structures of T cells that interact with H-2-incompatible cells may be allowed to differentiate according to the H-2 environment in which they circulate in tolerant conditions¹⁵. To accommodate the crossing of the H-2 barrier by LCMV or vaccinia immune T cells of chimaeras, interaction structures on these T cells for the tolerant H-2 haplotype could, theoretically, have undergone similar adaptive differentiation. Such a mechanism, however, in addition to the complex genetic controls previously mentioned^{11,12} seems to be very complicated. The concept that cytotoxic T cells are specific for altered cell-surface markers coded in *K* or *D* of H-2, and that T helper cells may be specific for antigen-altered cell surface structures coded in the *I* region, seems to be the simplest and most generally applicable explanation. Nevertheless, the physiological interaction idea cannot be disregarded until either altered *K*- or *D*-coded structures or physiological interaction structures are defined biochemically.

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Cytotoxic T lymphocytes recognise allogeneic tolerated TNP-conjugated cells

DURING a cytotoxic response, T cells can recognise and react to cell-associated virus antigens¹, minor histocompatibility

antigens^{2,3} or trinitrophenyl(TNP)-conjugated cell-surface components⁴ only when responder, stimulator and target cells share either K- or D-region determinants of the major histocompatibility complex (MHC). Two possible explanations for these observations have been discussed: first, cytotoxic T cells recognise non-H-2 antigens but to lyse targets they have to interact with self antigens controlled by the MHC on stimulator and target cells (cell-interaction determinants), and second, T-cell clones recognise and react to new antigenic determinants created by the association of MHC gene products with non-H-2 antigens. We present evidence here that T cells which have matured in a semi-allogeneic environment can recognise, in a specific way, TNP in association with tolerated allogeneic cells not sharing *H-2K* or *H-2D* loci with the responding cells.

DBA/2 T cells, tolerant to CBA/J alloantigens were obtained from chimaeric mice, produced by injecting anti-θ-treated DBA/2 bone marrow cells into 900-R irradiated F₁ (CBA/J×DBA/2) hybrids (DBA → CBA F₁ chimaeras)⁵. Five months after irradiation, chimaeric mice as well as (CBA/J×DBA/2) F₁ hybrids were immunised with TNP-conjugated CBA spleen cells (TNP-CBA). TNP was conjugated to either spleen cells or ⁵¹Cr-labelled LPS blasts according to a slight modification of the method of Shearer *et al.*⁴, using a cacodylate buffer, pH 7.3, containing 10 mM 2,4,5-trinitrobenzene sulphonate (1 ml per 10⁶ viable nucleated cells).

The *in vitro* assay of cell-mediated lympholysis (CML)⁶ as well as the assay for testing the lymphoid cell chimaerism of bone marrow chimaeras⁷ have been described before.

Using H-2^k anti-H-2^d and H-2^d anti-H-2^k sera the lymphoid cell chimaerism of DBA → CBA F₁ chimaeras was tested. As Table 1 shows, all cells from lymph nodes were killed by anti-H-2^d sera whereas no killing above background was observed with anti-H-2^k sera, indicating the absence of F₁ hybrid host cells.

Table 1 Killing of cells

	Incubation with		
	Anti H-2 ^k + C'	Anti H-2 ^d + C'	Saline + C'
Lymph node cells			
DBA/2	5%	97%	8%
(CBA×DBA/2) F ₁	83%	96%	9%
Chimaera CBA → DBA F ₁	8%	97%	9%

To test the reactivity of cells from DBA → CBA F₁ chimaeras as well as from (CBA/J×DBA/2) F₁ hybrids in CML, spleen cells were prepared and restimulated with either TNP-CBA stimulators or stimulated with SJL (H-2^b) cells *in vitro*. After 5 d, cultures were tested for cytotoxicity directed against TNP-CBA, CBA, TNP-DBA/2 or SJL ⁵¹Cr-labelled target cells. (CBA/J×DBA/2) F₁ cells restimulated *in vitro* with TNP-CBA lysed TNP-CBA targets (Fig. 1a) but neither CBA, TNP-DBA/2 nor SJL targets (Fig. 1b–d). The same responder cells stimulated *in vitro* with SJL cells lysed SJL targets but not CBA targets and to some extent also TNP-CBA and TNP-DBA/2 targets. This apparently nonspecific lysis of TNP-conjugated targets by cell suspensions containing high levels of cytotoxic activity has been found consistently, regardless of the strain combinations used. This is probably caused by nonspecific adherence of cytotoxic T cells to TNP-coupled targets. The assumption is supported by the fact that TNP-CBA-induced cytotoxic lymphocytes do not lyse non-conjugated target cells as SJL target cells (Fig. 1d). A result basically similar to that with TNP-CBA-primed F₁ responder cells was obtained with spleen cells from a DBA → CBA F₁ chimaera. Restimulation with TNP-CBA *in vitro* induced cytotoxic activity directed against TNP-CBA (Fig. 2a) but neither CBA, TNP-DBA/2 nor SJL targets (Fig. 2b–d). Stimula-

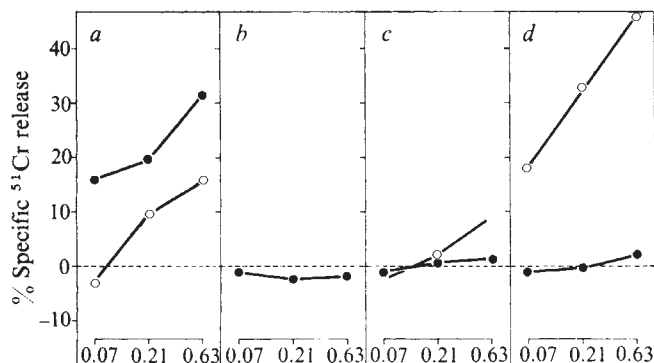
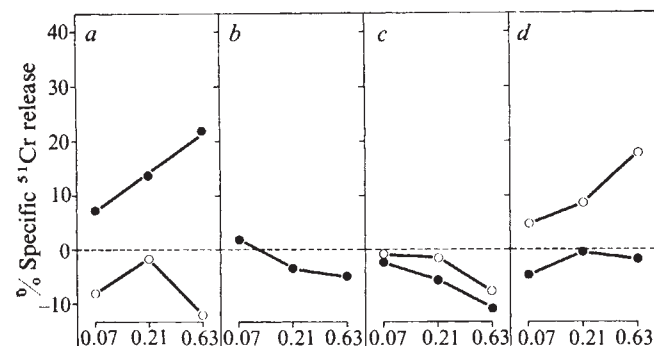


Fig. 1 Killing of TNP-CBA (a), CBA (b), TNP-DBA/2 (c) and SJL (d) targets by CBA $\times$ DBA F_1 spleen cells immunised *in vivo* with TNP-CBA spleen cells and restimulated *in vitro* with either TNP-CBA (●) or SJL (○) stimulating cells. The abscissa shows the known number ($\times 10^6$) of responders cultured on day 0, the descendants of which are the unknown number of killer cells added to each well containing 2×10^4 target cells. Each point represents the arithmetic mean of triplicate cultures. The standard deviation is less than 3%. The spontaneous ^{51}Cr release of the various targets varied between 20 and 31%.

tion with SJL cells resulted in lytic activity directed against SJL targets only (Fig. 2). On a per cell basis cytotoxicity of chimaeric cells compared with F_1 hybrid cells was lower on all targets. This is probably why nonspecific killing of TNP-coupled target cells was not seen.

Our experiments confirm conclusions drawn from work done using different experimental conditions: Doherty and Zinkernagel^{8,9} as well as Shearer *et al.*⁴ showed that F_1 hybrid cells sensitised to virus-infected or TNP-conjugated cells of one parental strain would only lyse 'modified' cells of that parental strain but not of the other. In addition it was shown that either H-2D or H-2K homology between modified stimulator and target cells was sufficient for generation of cytotoxicity. According to the hypothesis postulating the essential role of some MHC homology between effector and target cells for killing of modified targets one would have to postulate two separate 'cell interaction loci' which would have to be under allelic exclusion. This unlikely hypothesis can be rejected on the basis of the experiments reported here, which indicate that homology of MHC products between effector and target cells is not required to obtain killing of TNP-conjugated target cells, whereas homology of MHC products between TNP-conjugated stimulator and target cells is essential. Our experiments thus show that precursors for cytotoxic T cells are triggered and react to antigenic determinants created by association of

Fig. 2 Killing of TNP-CBA (a), CBA (b), TNP-DBA/2 (c) and SJL (d) targets by spleen cells from a DBA $\rightarrow$ CBA F_1 chimaera immunised *in vivo* with TNP-CBA cells and restimulated *in vitro* with either TNP-CBA (●) or SJL (○) stimulating cells. Figures on the abscissa indicate the number ($\times 10^6$) of responders per well (see Fig. 1).



MHC gene products with non-H-2 antigens (often referred to as 'altered H-2 antigens'). In what way these determinants are formed and recognised by T cells is unknown, and the subject of speculation^{10,11}.

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Binding of human immunoglobulins to pituitary ACTH cells

WE report the unexpected finding that the adrenocorticotrophic hormone (ACTH)-secreting cells of the human anterior pituitary gland show an affinity for human immunoglobulins resembling that of normal mast cells for IgE. This phenomenon could have physiological significance if considered in relation to the messages leading to synthesis or release of the hormone.

In the course of studies which led to the identification of antibodies directed against the prolactin-secreting cells of the adenohypophysis in patients suffering from autoimmune polyendocrine deficiencies¹, it was found that most human sera exhibited a cytoplasmic immunofluorescence (IFL) on ACTH-secreting cells. The type of endocrine cell was identified by a double IFL sandwich technique in which the patients' sera were followed by an anti-immunoglobulin conjugated with fluorescein isothiocyanate (FITC)(green) and a rabbit antibody specific for ACTH counterstained with a rhodamine-labelled conjugate (red) on the same section. None of the other five types of anterior pituitary cells reacted in this way, and anti-human Ig conjugates applied by themselves did not produce any fluorescence on ACTH cells. The three main immunoglobulin classes in human serum were reactive, although the strongest staining was usually seen with anti-IgM conjugates.

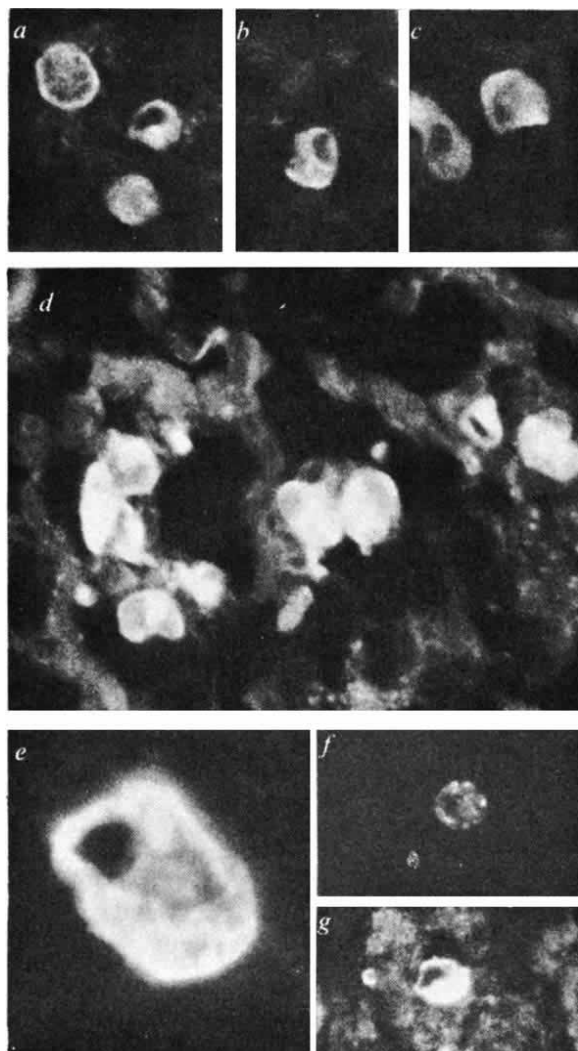
Experiments carried out to investigate this phenomenon suggested that the binding occurred through the Fc portion of the immunoglobulins and was not an antigen-antibody reaction. Table 1 shows the Ig preparations applied in these experiments. Four separate pools of mixed human Ig stained the ACTH cells. Two preparations of Fc fragments² from separate Ig pools were fully reactive, whereas the Fab from the same pools gave negative results using appropriate conjugates. One Fc and one Fab preparation made from normal pooled IgG failed to stain ACTH cells, indicating that the binding of IgG is of relatively low affinity and requires aggregates which cannot form with Fc fragments. A similar situation exists in relation to Fc receptors on lymphocytes. The strongest IFL was obtained with IgM in all categories of sera tested. Weaker staining was generally seen with IgG and IgA. One serum containing 30,000 IU IgE ml⁻¹ and several sera with normal IgE content gave negative results when tested with an anti-IgE conjugate. One IgD myeloma serum similarly failed to react when tested with anti-IgD. The staining of ACTH cells was positive when fresh human sera were followed by an anti- β_2 conjugate, showing that the binding Fc region was com-

Table 1 Analysis of Ig binding to pituitary ACTH cells

No. of preparations used	Type of Ig preparation	Antiserum (conjugated with FITC)	IFL on ACTH cells
4	Pools purified H-Ig (Cohn fraction II)	Sheep anti-H-F(ab) ₂ (laboratory preparation)	+
1	Laboratory preparation		
3	Lister Institute preparation		
2	F(ab) ₂ fragments from pooled H-Ig	Goat anti-H-Fc (Nordic)	+
1	F(ab) ₂ fragments from pooled H-Ig	Sheep anti-H-F(ab) ₂ (laboratory preparation)	—
2	Fc fragments from pooled human Ig	Sheep anti-H-F(ab) ₂ (laboratory preparation)	—
		Goat anti-H-Fc (Nordic)	+
1	Fc fragments from pooled H-IgG	Sheep anti-H-Fc (Wellcome)	+
1	Purified μ chain (μ -chain disease) (Dr E. C. Franklin)	Goat anti-H-Fc γ (Nordic)	—
1	Whole serum (μ -chain disease)	Goat anti-Fc μ (Nordic)	—
1	Monoclonal IgM- κ , 19S fraction	Sheep anti-Fc μ (Wellcome)	—
1	19S fraction of NHS	Goat anti-Fc μ (Nordic)	+
		Goat anti-Fc μ (Nordic)	+

plement fixing. Most sera showed titres between 1 and 80. Sera from cases of Waldenström's macroglobulinaemia were applied to determine whether the staining was dependent on any special features of the IgM tested. One of these sera gave a titre greater than 10,000 and the purified 19S fraction was equally reactive. This high reactivity, together with the fact that all anti-human Ig conjugates were effective—although they were raised in different animal species

Fig. 1 Human pituitary stained by immunofluorescence with normal human serum (Fc binding). *a-d*, Pituitary from Cushing's disease. Crooke cells show variable cytoplasmic staining. *e*, Single Crooke cell at high magnification. *f*, Live ACTH cell showing interrupted membrane fluorescence. *g*, Normal post mortem pituitary with small ACTH cell.



injected with separate pools of Ig—excludes the possibility that this IFL is due to a serum protein other than an immunoglobulin to which the animals might have developed adventitious antibodies. Further evidence is provided by the positive β_{1c} reaction and the negative tests obtained with most hypogammaglobulinaemic sera.

All human pituitaries showed this ACTH cell fluorescence, including six post mortem glands, and seven hypophysectomy specimens, five from carcinoma of breast cases and two removed for Cushing's disease. The reaction was still positive after fixation of the pituitary with acetone or methanol. Animal pituitaries are being tested but no conclusive results can as yet be given.

On unfixed cryostat sections of normal pituitary the ACTH cells were small with eccentric nuclei, and showed a uniform smooth staining pattern involving the entire cytoplasm (Fig. 1g). In Cushing glands containing numerous Crooke cells, the staining was intense and of variable pattern (Fig. 1a-e). When a suspension of mixed live pituitary cells was examined, normal sera gave an interrupted membrane fluorescence on cells which, after fixation, could be shown to react with anti-ACTH by the double IFL technique (Fig. 1f).

Results obtained with 430 individual human sera are shown in Table 2. All were first tested with anti-IgM and negative sera were retested with anti-IgG and anti-IgA; only those failing to stain ACTH cells with all three conjugates were listed as negative.

As might be expected, seven of the eight hypogammaglobulinaemic sera (supplied by Professor J. Soothill and Dr A. D. B. Webster) were completely negative. A weakly positive reaction was seen with one serum containing 13.9 IU IgM ml⁻¹. Seventeen normal sera from laboratory staff all stained ACTH cells but five healthy relatives belonging to a family with two cases of idiopathic hypoparathyroidism were surprisingly negative; and it is notable that 6 out of 15 hypoparathyroid sera also failed to stain these

Table 2 Binding to ACTH cells of human pituitary by the immunoglobulins in pathological and normal sera

Diagnosis	No. of sera tested	Negative fluorescence of ACTH cells
Hypopituitarism	20	3
Addison's disease	112	14
Hypoparathyroidism	15	6
Rheumatoid arthritis	80	11
Hypogammaglobulinaemia	8	7
Waldenström's macroglobulinaemia	31	2
Other conditions	143	4
Normal subjects	22	5*
Total	430	52 (12%)

* Normal relatives of hypoparathyroid cases.

cells, although they were not hypogammaglobulinaemic. Among the other categories of patients tested, the percentage of negative reactors varied from 3 to 14%.

From these data we conclude that certain sites within the Fc region of immunoglobulin molecules have a non-immunological affinity for an unknown constituent present both on the cell membrane and in the cytoplasm of ACTH cells in the human anterior pituitary. This was an unexpected finding and its physiological significance is not yet understood.

The existence of Fc receptors on subpopulations of blood lymphocytes³ and on mast cells and basophils, is established⁴. The fixation of IgE and of anaphylactic IgG on the mast cells by their Fc portions is necessary before the 'allergen challenge' stage of histamine release. This consists of attachment of the appropriate antigen and bridging of the free Fab portions of the Ig molecules; this process triggers the cells to release their granules. In the same way macrophages can be activated when immune complexes become attached to Fc receptors. Possibly a similar role might be found in future for the Fc receptors on ACTH cells. Note that Stanworth⁵ has shown that a sequence of basic amino acid residues within the ACTH molecules is capable of inducing histamine release from normal mast cells in the absence of immunoglobulins.

For the present it is important to be aware of ACTH-cell IFL when looking for autoantibodies to anterior pituitary gland in human patients. Furthermore this reaction may be used to identify ACTH cells for histological purposes, because the Ig binding seems to be as specific for the identification of these cells as for anti-ACTH sera raised in animals. It will also be of interest to determine whether lung cancers and other tumour cells with inappropriate ACTH secretion show this affinity for immunoglobulins.

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Infection of a cell line of mouse L fibroblasts with scrapie agent

THE infection of cell cultures with the causative agents of subacute spongiform encephalopathies—scrapie, kuru, Creutzfeldt-Jakob disease and transmissible mink encephalopathy—has not yet been reported. The development of a cell line (SMB), from the brain of a mouse affected with scrapie, in which the agent multiplies has shown that replication *in vitro* is possible¹. Studies with this cell line have, however, been limited because no control culture was available for comparative studies. This limitation has now, in part, been overcome by the successful infection of cultures of mouse L cells with scrapie agent, as described here.

The scrapie agent is closely associated with cellular fragments and attempts to release and purify the agent have

been unsuccessful. Recently Millson *et al.*² have shown that approximately 98% of the membrane proteins from scrapie membrane preparations can be solubilised by treatment with lysolecithin, giving an increase of up to 100-fold in the concentration of the agent. It seemed worthwhile to attempt to infect cells with this partially 'purified' preparation because, first, the removal of a large proportion of the membrane components made it possible to introduce a higher concentration of agent into the cell cultures than previously, and second, as lysolecithin can cause cell fusion³, it seemed possible that the association of bound lysolecithin with the particulate membrane fragments containing the scrapie agent might induce uptake of these fragments by the cells.

A membrane fraction was prepared from the brains of scrapie-affected mice by homogenising the tissue in 0.32 M sucrose to give a 10% suspension. Nuclei and unbroken cells were pelleted by centrifugation at 500g for 10 min. The post-nuclear supernatant fluid was centrifuged at 100,000g for 1 h and the sedimented membrane fraction was resuspended and homogenised in 25 ml of 0.05 M bicarbonate buffer, pH 8.3, containing 192 mg of lysolecithin. After incubation at 37 °C for 2 h, the sample was centrifuged at 100,000g for 1 h. The final pellet containing particulate membrane fragments was resuspended in 7 ml of Hanks' buffered salt solution. Dilutions of the suspension between 1/1 and 1/100 were then used to resuspend pellets of mouse L cells (derived from clone 929, Flow Laboratories) and the mixture was added to cell culture medium in medical flat bottles. The medium used was Eagle's minimum essential medium with 10% foetal calf serum.

The lysolecithin-prepared suspensions of membrane fragments were toxic to the L cells but some survived and two cultures were established. Culture *A* was from cells exposed to a suspension diluted 1/3 and culture *B* was from dilution 1/100. Monolayers were eventually obtained in each case about 6 weeks later. The monolayers were then submitted to 15 serial passages during a period of up to 29 weeks and on each occasion dilutions of the cells were made equal to 10^{-6.5}.

The presence of scrapie activity at the 1st, 7th, 11th and 15th passages was determined by titration of samples of the cultures in mice as described before⁴ (Table 1). In culture

Table 1 Titre of scrapie agent in two cultures of mouse L fibroblasts at various passages after exposure

Culture	Passage			
	1	7	11	15
<i>A</i>	3.3*	1.6	1.0	1.8
<i>B</i>	1.8	1.6	1.5	1.1

* Titre of scrapie agent log₁₀LD₅₀ per 0.05 ml.

A there was a decline in titre from the 1st (3.3 log₁₀ LD₅₀ per 0.05 ml) to the 7th (1.6) passage and thereafter the titres remained at about the latter level. In culture *B* the titres varied between 1.8 and 1.1 from the first to the 15th passage. These titres correspond to about 3,000 cells per LD₅₀, which is a much lower level of infectivity than for the SMB cell line (100 cells per LD₅₀). The difference between the titre of the first passage of cultures *A* (3.3) and *B* (1.8) is the same as the difference in the dilutions of suspension used for the exposure of cells to scrapie infectivity. This suggests that at least some of the activity of the first passage was derived from the suspension of membrane fragments and not from multiplication of agent in the culture.

The levels of scrapie activity in the original mixtures of membrane suspension and L cells were estimated at about 10^{3.5} (*A*) and 10^{4.0} LD₅₀ per 0.05 ml (*B*) so that by the 12th and 9th passages, respectively, the original infectivity, if it had all been retained in the cultures at the first passage, would have been eliminated by the subsequent dilution of

the cells at each passage. The presence of activity at later passages must, therefore, be due to multiplication of agent. Further, since each passage involved a dilution of cells, the observation of a similar level of activity at each passage titrated also indicated that the agent had multiplied.

Fusion of plasma membrane can occur as a consequence of exposure of cells to lysolecithin, and multinucleated forms may be seen³. During the development of the initial monolayers of L cells exposed to scrapie agent an increased number of multinucleated cells was noted, suggesting that fusion had occurred. The establishment of cultures in which the scrapie agent was replicating could have been due to the presence of lysolecithin which, bound to membrane fragments containing scrapie infectivity, fused with the L cells. Fusion could also have resulted from the presence of free lysolecithin in the membrane suspension used to infect the cultures. Scrapie infectivity is associated with plasma membrane of SMB cells⁴; the successful infection of cell cultures in the presence of a substance known to cause fusion of membranes is consistent with the observation that the agent is located on the membrane.

Although the titres obtained in the cultures of L cells were low, the evidence that infection of cell cultures with scrapie agent has occurred is encouraging. It suggests that further attempts to fuse membrane fragments from scrapie-affected tissue to cells should be made.

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Renal tubular adaptation to dietary phosphorus

THE kidney is very important in the maintenance of the overall balance of inorganic phosphate (P_i). The renal response to variations in dietary P_i has been generally explained by changes in the amount of P_i filtered by the glomerulus. When a change in the tubular P_i transport has been suspected, it has usually been attributed to an alteration in parathyroid hormone (PTH) secretion^{1,2}. We have shown that the renal tubule changes its capacity to reabsorb P_i according to homeostatic requirements independently of the filtered load of P_i (ref. 3). Since such a tubular adaptation also occurs in the absence of PTH (ref. 3), the question of the relative potency of PTH and that of dietary P_i in changing the tubular transport of P_i is of interest. Here we report that variations in dietary P_i cause a much greater change in the tubular capacity to transport P_i than removal of the thyroparathyroid glands.

The renal handling of P_i was studied in pairs of sham-operated (SHAM) and thyroparathyroidectomised (TPTX) rats fed diets containing either low (0.2 g % dry weight) or high (1.8 g % dry weight) amounts of phosphorus for ten days (see Table 1 legend). The fractional excretion of P_i ($C_{P_i}/C_{In} \times 100$), measured in steady-state conditions and at the same plasma levels of P_i ($[P_i]_{P_i}$), was about five times higher in SHAM than in TPTX rats on a high P_i diet (Table 1). As expected, calcaemia ($[Ca]_{P_i}$), measured during the clearance periods, was higher in SHAM than in TPTX animals (2.43 ± 0.04 and 1.78 ± 0.16 mM respectively, $P < 0.01$). We found no significant differences in the weight of the animals (182 ± 3 and 174 ± 5 g), in urinary volume (37 ± 5 and 30 ± 3 μ l

Table 1 Variation in the fractional excretion of P_i in response to dietary P_i compared with thyroparathyroidectomy

Diet	$C_{P_i}/C_{In} \times 100$		
	SHAM	TPTX	SHAM-TPTX
High P_i (1.8 g % dry weight)	34.7 ± 4.4 (15)	7.1 ± 1.8 (7)	4.9
Low P_i (0.2 g % dry weight)	0.098 ± 0.003 (7)	0.045 ± 0.009 (7)	2.2
High/low P_i	354	157	

Numbers in parentheses correspond to number of animals. Fractional excretion of phosphate ($C_{P_i}/C_{In} \times 100 \pm$ s.e.m.) was measured at equal plasma phosphate concentrations in pairs of rats fed high P_i (1.8 g % dry weight) and low P_i (0.2 g % dry weight) diets for 10 d. The mean ($\pm$ s.e.m.) intake of phosphorus was 31 ± 1 and 276 ± 13 mg d⁻¹ for rats fed on a low and high P_i diet respectively. Dietary Ca^{2+} and Na^+ contents were kept constant (1.2 and 2.45 g % dry weight respectively). Surgical thyroparathyroidectomy (TPTX) or a sham operation (SHAM) was performed on the eighth day. The effectiveness of parathyroidectomy was evaluated after the clearance experiment; the rats were returned to the lab chow for 5 d, fasted overnight and their calcaemia ($[Ca]_{P_i}$) determined the following morning by atomic absorption spectrometry. Data are only presented for TPTX rats which displayed a $[Ca]_{P_i}$ below 1.88 mM ($= 7.5$ mg %) in these conditions and which were therefore considered to be fully parathyroidectomised. On the day of the clearance measurement animals were infused intravenously (4 ml h⁻¹) with either isotonic NaCl or neutral P_i (16% NaH_2PO_4 and 84% Na_2HPO_4) at doses of 60 or 120 μ mol h⁻¹. The osmolality of the solutions was adjusted to 300-310 mosmol l⁻¹ by addition of NaCl where necessary. The clearances were measured in non-fasted conscious animals according to a technique described earlier⁴. Inulin was determined by the anthrone technique⁵ and P_i by the method of Chen *et al.*⁶. Data were analysed only from values of $[P_i]_{P_i}$ in the range between 2.25 and 2.90 mM. The mean $[P_i]_{P_i}$ values of the four experimental groups were not significantly different: high P_i diet, SHAM 2.54 ± 0.01 and TPTX 2.60 ± 0.07 mM; low P_i diet, SHAM 2.46 ± 0.09 and TPTX 2.49 ± 0.11 mM.

min⁻¹), or in C_{In} (1.29 ± 0.06 and 1.33 ± 0.04 ml min⁻¹). Similar results were obtained in SHAM and TPTX rats, on a low P_i diet (Table 1). The ratio C_{P_i}/C_{In} was twice as high in SHAM as in TPTX animals. $[Ca]_{P_i}$ was higher in SHAM than in TPTX rats (2.46 ± 0.05 and 2.00 ± 0.09 mM, $P < 0.001$). Again there were no significant differences in weight (170 ± 5 and 169 ± 4 g), in urinary volume (38 ± 6 and 40 ± 6 μ l min⁻¹), or in C_{In} (1.33 ± 0.07 and 1.29 ± 0.10 ml min⁻¹). In addition, none of these latter parameters differed significantly from those of the two groups on high P_i intake.

Thus, independently of the filtered load, and according to the values of the SHAM-TPTX ratio in Table 1, the presence of PTH may increase the fractional excretion of P_i about 2-5 times. The greater effect due to the removal of the parathyroid glands under high P_i diet can be explained by parathyroid hyperfunction, occurring in this condition². The fractional excretion of P_i can, however, be changed more than a hundredfold in both intact and TPTX rats by varying the previous dietary intake of P_i as expressed by the ratios of fractional clearances on the high and low P_i diets (Table 1).

The dietary P_i level, therefore, seems to control a mechanism which is independent of circulating PTH and which seems to be much more potent than this hormone in changing the renal tubular transport capacity of P_i . The present data indicate that PTH would amplify the important dietary-induced change in the tubular capacity for this anion by enabling more P_i to be excreted under a high P_i diet.

Differences induced by dietary input of P_i can be observed in the presence of the same calcaemia (see above) and the same urinary pH (ref. 3). It is maintained under marked expansion of the extracellular volume³. Renal adaptation to P_i is probably independent of vitamin D₃ and its metabolites since in TPTX vitamin D-deficient rats maintained on a low P_i diet⁷, tubular reabsorption of P_i is complete.

Tubular adaptation to dietary P_i seems not to be mediated by the main factors generally believed to be most important in the regulation of the renal handling of P_i . Our results have

revealed a homeostatic mechanism of great potency which must contribute to the maintenance of P_i balance by the kidney.

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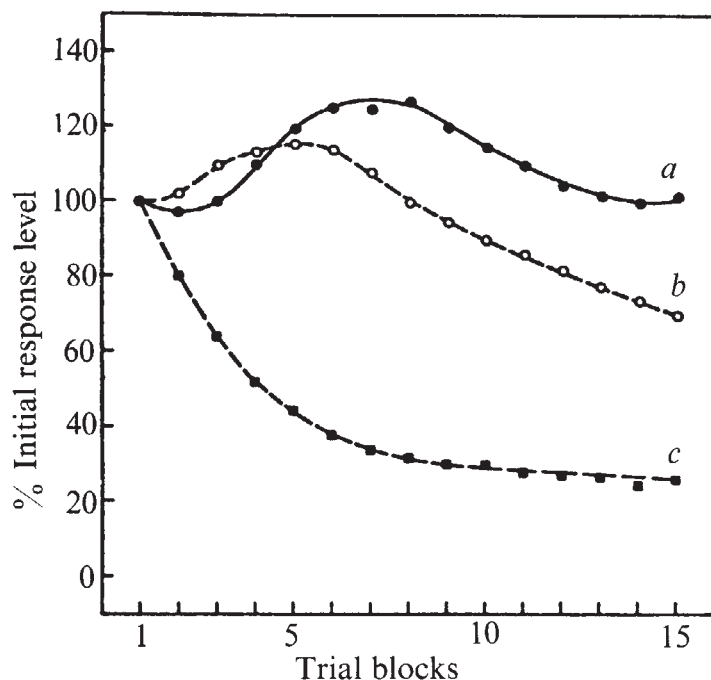
Computer simulation of a model of habituation

HABITUATION—in general defined as a reversible decrement of response to repeated stimulation—is a widely studied form of behavioural plasticity. The reversibility sets habituation apart from fatigue and accommodation. An habituated stimulus may once again be able to evoke a response after the presentation of an intervening novel stimulus. This phenomenon, known as dishabituation, rules out sensory and motor fatigue as the bases of the response decrement.

Thompson and Spencer¹ have described several characteristics which together form a widely accepted operational definition of habituation. Two of these are that relative response decrement, expressed as a percentage of the unhabituated response, proceeds more rapidly for less intense stimuli, and that both habituation and dishabituation recover spontaneously over time if the stimulus is withheld.

Although habituation has been studied at the neurophysio-

Fig. 1 Curves of relative habituation in cat spinal cord, taken from ref. 6 with permission of the authors and publisher (their Fig. 7a). Stimulus strength: a, strong; b, moderate; c, weak.



logical level in a number of preparations, in most cases the mechanism underlying the response decrement is unknown. Several circuit models based on plausible theories of habituation have been constructed to help to interpret experimental results and to explore possible habituation mechanisms²⁻⁵; such models can help identify the requisite properties of decremental and incremental effects within the framework of a particular theory. With this in mind, I have explored, by mathematical analysis and computer simulation, a circuit model based on Thompson's two-process theory of habituation^{5,6}. This theory was chosen as the basis of the work because of its wide applicability and acceptance in physiology and psychology. I show here how the effects of stimulus intensity can be realised by a circuit model similar to one proposed by Groves and Thompson⁶.

In Thompson's two-process theory, response strength is determined by the sum of transmissions in two independent sets of channels from stimulus to response. Transmission decrements occur in direct stimulus-response channels, leading to observed response decrements at low stimulus intensities. Transmission increments, or sensitisations, occur at higher stimulus intensities in indirect channels associated with the overall state of reactivity of an organism. Dishabituation, then, is a transient facilitation superimposed on a depressed response channel following presentation of a novel, usually strong, stimulus. During habituation training, the balance between decrement and sensitisation produces a response that is stronger or weaker than the control (unhabituated) response. This balance shifts with the number of presentations at a given stimulus intensity to generate response curves of the form shown in Fig. 1, which were derived from experiments on the handlimb flexion reflex of spinal cats⁶. At low intensities the decrement prevails, whereas at higher intensities sensitisation causes the response to rise above its control level in early trials. Thompson⁶ has illustrated the two-process theory with a circuit model which is proposed for cat spinal cord. In this circuit, stimulation results in synaptic depression in the direct stimulus-response path, and in synaptic increment or enhanced cell excitability in the indirect path, or state portion.

My model circuit (Fig. 2) is a simplification of Thompson's model. The circles represent cells or cell populations (referred to here simply as 'cells'). Cell H receives the external stimulus from its afferent, I. The output of cell O represents the circuit's overall response. The direct connection from H to O represents the direct stimulus-response channel. Cell S is the indirect path, or state system, of the circuit, and the channel from H to O through S represents the effect of this state system on the overall output. The output of each cell is a weighted sum of its inputs; that is

$$H = w_0 I \quad (1)$$

$$S = w_1 H \quad (2)$$

$$O = w_2 H + w_3 S \quad (3)$$

The value of each cell's output at a given moment may be interpreted either as the instantaneous firing rate of a single cell or as the number of cells in a population that are then firing. The value of the external stimulus may be thought of similarly. The value of each coupling weight may be regarded as the strength of the synapse connecting the two corresponding cells or as the effective synaptic transmission strength between two populations.

For the direct stimulus-response channel to become less effective with use, the coupling weight w_2 must become smaller on repeated stimulation. Similarly, the compound effect of w_1 and w_3 must increase with use to represent the sensitisation process. The influence of I on H does not change with stimulation, so w_0 is fixed. Changes in w_2 must occur so as to generate responses that yield a negative exponential function of the number of stimulus presentations. Further, the strengths

of all coupling weights must tend towards their initial values in the absence of stimulation of the system. These requirements can all be met if each weight is modelled with a first-order differential equation which is driven by the output of the associated presynaptic cell. So

$$T_i \dot{w}_i = w_{i0} - w_i + a_i F_i(X); \quad i = 1, 2, 3 \quad (4)$$

where $\dot{w}_i$ denotes the time derivative of the associated weight value, and

$$X = \begin{matrix} H & \text{for } i = 1, 2 \\ S & \text{for } i = 3 \end{matrix} \quad (5)$$

For each i , w_{i0} is the initial value of the weight and the resting level towards which it moves when stimulation stops. The rate of return, which represents the rate of spontaneous recovery of habituation and sensitisation, is determined by the time constant, T_i . The functions F_i determine the way each weight is affected by its modifying input, X , and are non-negative for all values of X . If a_i is negative, the corresponding weight will be decreased by stimulation of its associated presynaptic cell; if a_i is positive, the weight will be increased.

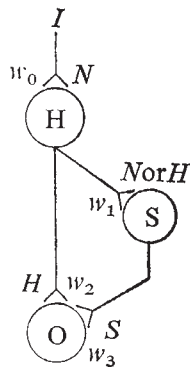


Fig. 2 Two-process circuit. N , non-plastic synapse; H , depleting synapse; S , sensitising synapse.

To determine the modification function for each weight, note first that sensitisation is a direct function of stimulus intensity⁶, for now I suppose that $F_3(S)$ is simply S itself, so that the rate of increase of w_3 varies linearly with the output of cell S . Thompson maintains that relative habituation due to the decremental process alone is independent of stimulus intensity⁶. This requires that the F_i associated with each decreasing weight be a constant—that is, each weight that decreases with use must be driven by a constant at each presentation of a stimulus, regardless of stimulus intensity. More detailed circuits have been designed that obviate the need for this assumption, but in the investigations reported here I have used the circuit of Fig. 2 and the assumption of constant synaptic modification drive.

There are two ways that the circuit operating under these assumptions can generate curves of relative habituation similar to those in Fig. 1. They are based on whether or not w_1 is allowed to vary with use. If w_1 is fixed and w_3 changes more rapidly than w_2 , at moderate and high stimulus intensities the overall response to repeated stimulation first increases across trials as w_3 increases, then falls as w_2 decreases. At low intensity, only the decrease in w_2 is significant, so the response only decreases across trials. Curves of relative habituation generated in computer simulation of this case are shown in Fig. 3. Note that in this case, since the output of cell S is constant across trials, sensitisation itself does not fall after rising, as required by Thompson's theory. The curves of Fig. 3 are qualitatively similar to those of Fig. 1, however, indicating that this assumption about sensitisation may not be required to generate such curves.

If w_1 is allowed to decrease with use, but more slowly than w_3 increases, sensitisation first increases, then decreases, across

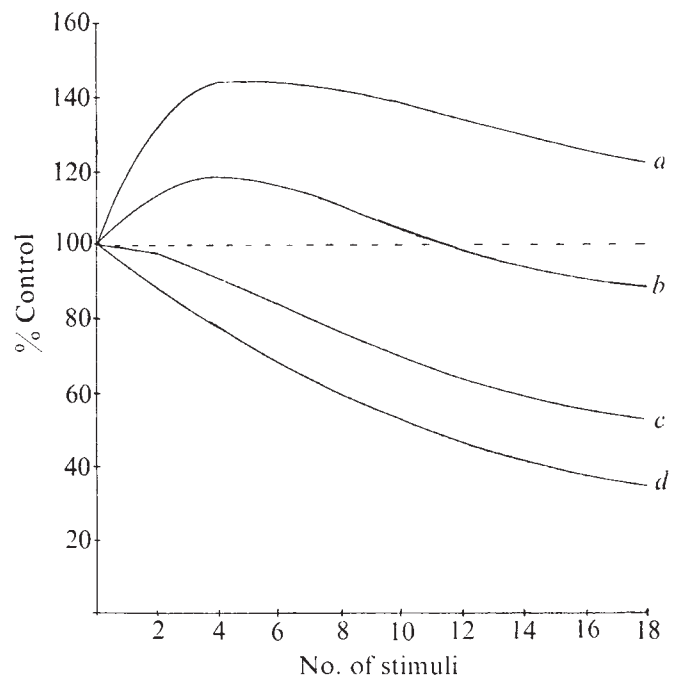


Fig. 3 Curves of relative habituation generated by computer simulation of the circuit of Fig. 2. Stimulus intensities: a , 8.0; b , 5.0; c , 2.0; d , 1.0.

trials as the output of cell S decreases. This effect alone can generate response curves like those of Fig. 1. When added to the strategy in which changes in w_2 and w_3 generate the desired response curves, a small drop in w_1 causes the effect of sensitisation to be reduced further in later trials. This decrease in w_1 allows greater variation in w_3 to produce more sensitisation in early trials but with a return closer to control levels in later trials.

Computer simulation of this simple habituation model has thus shown how the processes of decrement and sensitisation postulated in the two-process theory may vary with respect to one another to generate realistic response curves. Thompson's assumption that sensitisation decreases in later habituation trials has been shown to be unnecessary for the production of the intensity curves of Fig. 1. Such variation can lead to lower final response levels at higher stimulus intensities, however, and is necessary to explain habituation or dishabituation.

Further simulations have shown how the effects of stimulus frequency could arise in the model, and what effects nonlinear weight modifications could produce. A network using the circuit of Fig. 2 as a basic unit has also been simulated to investigate stimulus generalisation, and intensity-independent relative habituation has been produced by an augmented model that uses synaptic weights which decrease as a function of stimulus intensity. My simulations have provided insights into plausible functional organisations that may be difficult to investigate experimentally, and as such should contribute to the design of more decisive future experiments.

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Sprouting and regeneration of synaptic terminals in the frog cardiac ganglion

SYNAPTIC connections are capable of a remarkable proliferation after nervous tissue has been damaged and some synapses have been destroyed. This proliferation, or 'sprouting', of remaining, intact, synapses within partially denervated tissue has been found to occur in the peripheral, autonomic and central nervous systems¹⁻⁵. As yet, however, the functional ability of these sprouted synaptic contacts has not been extensively tested and the persistence of the sprouted synapses over long periods has not been studied. We have been investigating these problems in the parasympathetic cardiac ganglion in the frog. Earlier studies have shown that when the nerve supply to the ganglion is partially destroyed by crushing one vagus nerve, axons in the other vagus nerve sprout and synapse with the denervated neurones¹. Here I describe experiments in which the persistence of the sprouted synapses in the cardiac ganglion was tested by allowing the damaged axons to regenerate into the tissue. The results indicate that regenerating vagal fibres are able to re-establish some, but not all, of their synaptic connections and that innervation from the sprouted nerve decreases.

The cardiac ganglion in the frog is well suited for studying synaptic transmission at sprouting synapses. Because the ganglion is very thin, individual neurones can be seen in the living isolated preparation and this greatly facilitates intracellular microelectrode recording. Ganglion cells are grouped along two branches of the vagus nerves as they traverse the interatrial septum^{1,6}. Each branch contains preganglionic axons from both left and right vagus nerves since the branches are joined by a chiasma near the entry of the vagus nerves into the heart. Ganglion cells are mostly innervated by the ipsilateral vagus nerve. For example, along the left branch of the ganglion in control animals, 87% of the neurones receive inputs from the left vagus and 48% from the right (about one-third of the cells are innervated by both nerves)¹. Many ganglion cells are innervated by two or more preganglionic fibres; usually only one fibre produces suprathreshold excitatory postsynaptic potentials (e.p.s.p.s) and stimulation of the other fibres evokes subthreshold responses (Fig. 1a). After crushing the left vagus nerve, the remaining vagal axons sprout and within about 1 week, all the ganglion cells are innervated by the right vagus nerve¹. The experiments described below were carried out at longer intervals after crushing the nerve, to study regeneration and the fate of the sprouted synapses.

The experimental methods have been described previously¹ and will only be outlined briefly here. The left vagus nerve was crushed with fine forceps in frogs anaesthetised with 0.5% tricaine methanesulphonate (Sigma). The animals were allowed to recover for varying periods before synaptic transmission was tested. Neurones in the isolated cardiac ganglion were impaled with fine-tipped microelectrodes. E.p.s.p.s were recorded in response to stimulation of the right and/or left vagus nerves, which were drawn into separate suction electrodes.

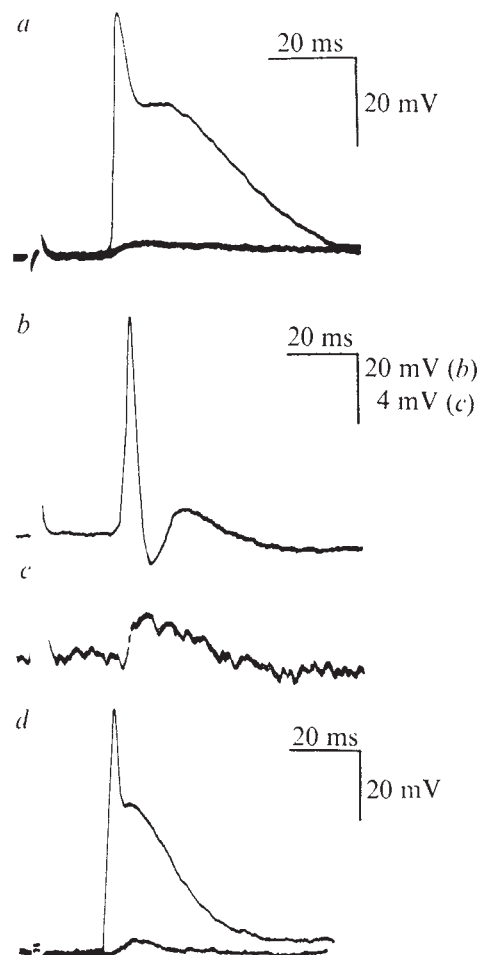
As the left vagus nerve regenerated, it re-established some synaptic connections. E.p.s.p.s produced by regenerating fibres tended to be subthreshold early in the course of the regeneration (4-8 weeks), (Fig. 1, b and c). Later, both sub- and suprathreshold e.p.s.p.s were evoked by stimulation of regenerating fibres, as in normal ganglia (Fig. 1d). In addition, as the regenerating fibres re-established

synaptic connections, there was a decline of innervation from the right vagus (Fig. 2). Experiments are in progress to determine whether a comparable decline of innervation from the right vagus nerve occurs when reinnervation is prevented.

At the longest recovery period studied, about 42 weeks, the innervation pattern of the ganglia was not restored to normal; the nerve supply to cells along the left branch of the ganglion was still dominated by axons from the right vagus nerve, in contrast with unoperated animals (Fig. 2). The regenerating fibres were unable to restore completely their original connections.

These data differ from the results of experiments in which the nerve supply regenerates to ganglion cells that are innervated by an implanted foreign nerve and not by sprouted synaptic terminals; Purves found that when the preganglionic supply to sympathetic ganglia (superior cervical ganglia) of guinea pigs is cut and the parasympathetic vagus nerve is rerouted into the ganglia, the vagal endings form synaptic contacts which are not displaced when the original innervation regenerates⁷. That is,

Fig. 1 Intracellular recordings from multiply-innervated parasympathetic neurones located on the left branch of the cardiac ganglion. *a*, Superimposed recordings from a ganglion cell in an unoperated animal. Threshold stimulation of the left vagus nerve evoked a subthreshold e.p.s.p. Stronger stimulation produced a suprathreshold response. *b* and *c*, Ganglion cell innervated by both the right vagus (*b*) and regenerated left vagus nerve (*c*). Stimulation of the left vagus produced a subthreshold e.p.s.p. The left vagus nerve had been crushed 41 d before the experiment. *d*, Ganglion cell with multiple innervation from the regenerated left vagus nerve. The left vagus had been crushed 302 d before the experiment. Weak stimulation of the regenerated nerve evoked a subthreshold e.p.s.p. Stronger stimulation produced a suprathreshold response, as in unoperated ganglia.



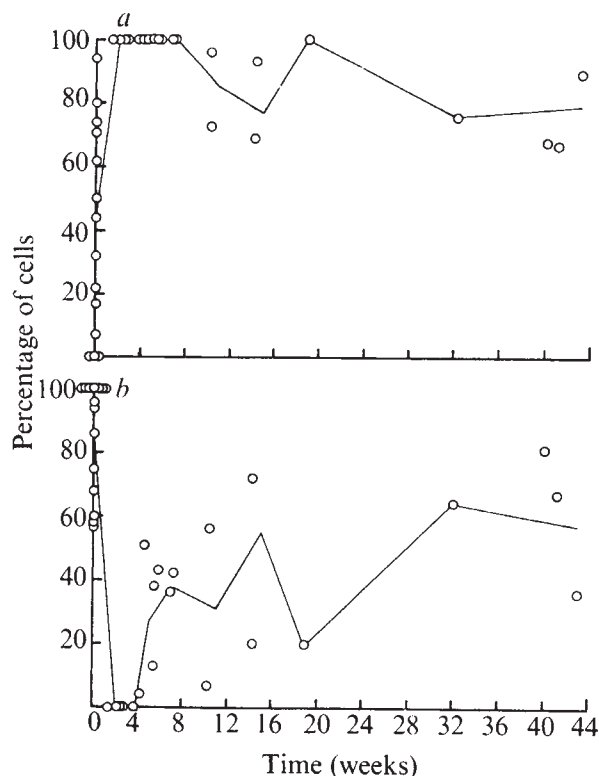


Fig. 2 Sprouting and regeneration of synapses in the cardiac ganglion. Each graph shows the percentage of cells located on the left branch of the ganglion which were innervated by the right (a) and left (b) vagus nerve at different periods after the left vagus nerve was crushed. Each circle represents data from one ganglion (15–30 cells recorded per ganglion). Circles on the ordinate show data from unoperated ganglia. A line has been drawn connecting the averages of data grouped into weekly intervals; the last three points were grouped together. Sprouting of synapses from the right vagus nerve is indicated by the increase of right vagal innervation from 48 to 100% within 1 week of the operation (a). Regeneration of left vagal synapses begins after about 4 weeks (b). At the first stages of regeneration (4–8 weeks) there did not seem to be any change in right vagal innervation (a), but this is misleading; slight decreases in the size of right vagal e.p.s.p.s are not indicated on this figure.

many of the sympathetic ganglion cells are innervated by both nerves. On the other hand, the present experiments confirm and extend to a cellular level the observations of Guth and Bernstein⁸ who studied the reinnervation of superior cervical ganglia of cats after partial deafferentation. They reported that after damaging thoracic sympathetic rami which contributed pupillodilator fibres, remaining intact preganglionic fibres sprouted and took over this function. When the preganglionic fibres regenerated, stimulation of the sprouted nerve no longer produced pupillodilation; reinnervation by the appropriate axons was accompanied by a decrease in the innervation arising from sprouted fibres.

The present experiments do not explain the mechanism by which innervation from the sprouted right vagus nerve decreases during regeneration of the left vagus nerve or even if the reinnervation causes this decrease. Because transmission at the sprouted synapses is indistinguishable from normal synapses¹, it is not possible to determine whether sprouted or original right vagal terminals are lost during regeneration. It is possible that right vagal synapses are 'suppressed' by the regenerating axons but are left structurally intact⁹. It is also possible, however, that a slow rate of spontaneous synaptic degeneration occurs¹⁰ and when a right vagal ending degenerate, axons from the left and right vagus nerve compete for the vacated site.

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Function of muscarinic and nicotinic acetylcholine receptors

ACETYLCHOLINE receptors in vertebrates can be classified in two categories—nicotinic and muscarinic—on the basis of differential sensitivity to agonist and antagonist compounds^{1,2}. This pharmacological distinction, although useful both in therapeutics and in experimental physiology, has no known biological significance. I wish to show here that there are marked kinetic differences between nicotinic and muscarinic receptors, and to suggest that these differences have a clear physiological meaning.

Two features are shared by all nerve-mediated muscarinic responses that have been studied electrophysiologically: the minimum latency is ~ 100 ms, and the duration after a single stimulus is 0.5 s or longer^{3–7}. In contrast, however, the synaptic delay at the skeletal neuromuscular junction may be only 0.22 ms (ref. 8), and even at the slowest nicotinic synapses, the synaptic potential lasts no more than 30–100 ms (refs 9 and 10). The discrepancy in the latencies cannot be explained by morphological differences between nicotinic and muscarinic junctions, although the separation of nerve and effector is more variable at the autonomic junctions than at the skeletal neuromuscular junction^{11,12}. Experiments in which acetylcholine (ACh) was applied iontophoretically to muscarinic receptors of cultured intestinal smooth muscle cells have shown that the long latency is post-junctional in origin¹³. This result has been confirmed in adult smooth muscle¹⁴ and in salivary glands¹⁵.

One circumstance in which a long slow response is of obvious importance is during vagal slowing of the cardiac pacemaker. This function would be ill served by an inhibitory mechanism operating at 'nicotinic speeds', for not only would most of the inhibitory input be ineffective, but the interval between beats would be critically dependent on the arrival of inhibitory impulses immediately before the projected time of discharge. There is little direct evidence that cardiac muscarinic receptors operate slowly, since estimates of the latency after single volleys have been made using preganglionic stimulation^{3,4,16}. The delay due to relay in the cardiac ganglia, however, is probably small¹⁷.

del Castillo and Katz⁴ applied ACh iontophoretically to the sinus venosus of the frog, and could duplicate the main features of the response to vagal stimulation. I have examined the onset of the response to close iontophoretic application of ACh in a preparation (interatrial septum of the frog *Rana temporaria*) which allowed observation of

the position of micropipettes with good phase contrast optics at a magnification of $\times 140$ or $\times 220$. The minimum latencies of intracellularly recorded responses (Fig. 1) were 200–300 ms at 24 °C and 400–550 ms at 15 °C. The minimum latency was affected little by lateral displacements (up to 100 μm) of the ACh pipette away from the recording site. It was also relatively insensitive to small (5–15 μm) vertical displacements. This behaviour parallels that seen in comparable experiments on smooth muscle¹³. Ganglion-blocking drugs (hexamethonium, 2×10^{-4} M; mecamylamine, 10^{-4} M) had no effect on the latencies. A striking feature of the responses was their long duration, brief pulses of 0.5–1 nC producing hyperpolarisation which could last for 8 s or more.

The effective stimulus presented to the tissue can be estimated from the diffusion equations, if some simplifying assumptions are made about the geometry. For example, ACh diffusing freely from a point source 10 ms in duration situated 10 μm above an infinite plane surface will reach its peak concentration immediately below the source in less than 30 ms. The concentration decline is also rapid, reaching 5% of the peak value by 400 ms. Since the muscle cells are interconnected electrically¹⁸, ACh acting at some distance from the recording site presumably also contributes. The average concentration, calculated as the concentration-area product integrated over the whole plane, is perhaps a fairer measure (Fig. 2). The onset of the response lags behind the stimulus. The effective diffusion distances, which might be longer than suggested by the model, are uncertain and there may be diffusion barriers. But the minimum distance between the ACh pipette and the muscle was certainly less than the value of 10 μm assumed for calculation, since in many experiments the tip of the ACh pipette could be seen to dimple the surface of an underlying muscle cell. These results support the conclusion that inhibitory muscarinic responses in the heart are at least as slow as excitatory muscarinic responses elsewhere.

The duration of the inhibition after a single vagal volley

Fig. 1 Intracellular electrical records from isolated interatrial septum of the frog, showing responses to iontophoretically applied ACh. *a*, Record from a spontaneously active preparation. The arrow shows the time of application of a 15-ms, 40-nA pulse of ACh. *b*, Initial phase of response in quiescent preparation at higher amplification and sweep speed, showing well defined latency. Temperature 15 °C. ACh pulse (10 ms, 70 nA) at arrow. Vertical calibration 50 mV in (*a*), 5 mV in (*b*). Time marker, 1 s in (*a*), 0.1 s in (*b*).

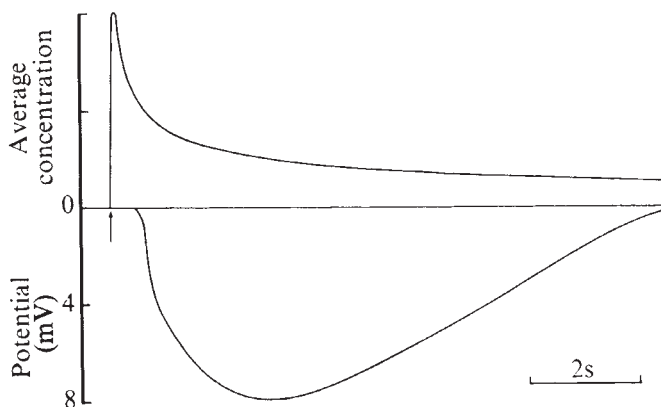
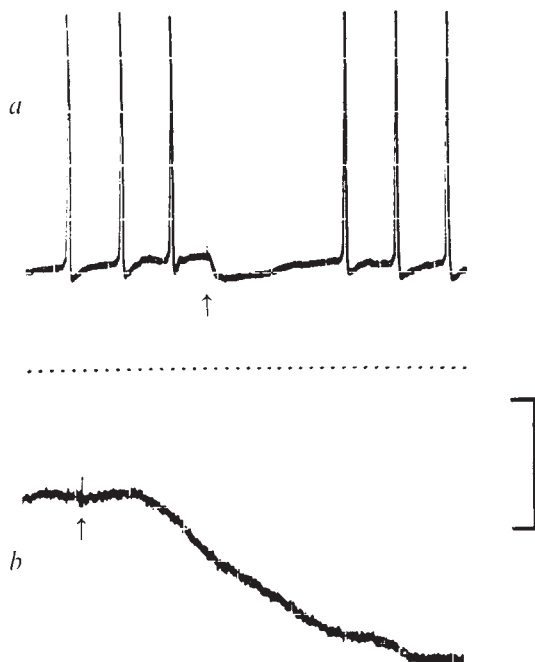


Fig. 2 Comparison of redrawn voltage response (lower curve) with calculated average concentration change of ACh. Upper curve calculated as $M(\pi Dt)^{-1} \exp(-x^2/4Dt)$, where M is amount released from instantaneous point source, D is the diffusion constant ($8.10 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$), x is the vertical separation between source and tissue (10 μm) and t is time. Vertical scale arbitrary. The expression is identical with that giving the concentration at distance x from a plane source. The error made in assuming instantaneous release in place of a 10-ms source is negligible at times greater than ~ 50 ms.

is 20 s in the turtle¹⁶, 5–7 s in the frog⁴ and 2–3 s in the cat³. Thus a single impulse has effects which extend over several cardiac cycles, facilitating temporal summation and continuity of successive responses, even at the low firing frequencies of autonomic nerves. It is probably no coincidence that animals with low heart rates show longer vagal responses. Conversely, at the skeletal neuromuscular junction it would be inappropriate to have provision for temporal summation, since smooth grading of contractions is obtained by other means. Nicotinic receptors provide the required 1:1 transmission with minimum latency and maximum temporal precision. If the argument given earlier is valid, the excitation produced by adrenergic nerves in the heart should be slow. This is indeed the case: the latency of sympathetic β cardioacceleration is several seconds and the effects of a short period of stimulation last for minutes^{19,20}. Responses of excitatory α receptors, for instance in the vas deferens, are some orders of magnitude faster²¹.

How are the slow responses mediated? There are indications that cyclic GMP acts as an intracellular 'second messenger' for muscarinic receptors^{22–24}, and there is strong evidence that cyclic AMP plays the same part for β receptors^{23,25}. The evidence from latencies and time courses is not at variance with these ideas.

I propose that muscarinic receptors are specialised to allow temporal summation and thus continuity of action, whereas nicotinic receptors are specialised to prevent it. The long latency, as such, at muscarinic junctions is of doubtful biological relevance. Nevertheless, it provides a convenient pointer to a physiologically significant slowness of response.

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Glutamate and quisqualate noise in voltage-clamped locust muscle fibres

GLUTAMATE-SENSITIVE receptors occur widely, both in vertebrate and invertebrate nerve and muscle membranes^{1,2}. Information concerning the action of agonists on glutamate receptors and the kinetics of the associated ion channels may be obtained from a study of the noise which results from conductance fluctuations during drug action³⁻⁸. Analysis of voltage-clamped noise has been used in the present work to investigate the lifetime of an open channel in the presence of a steady concentration of a chemo-excitant, and to obtain an estimate of the single-channel conductance. In addition, we have examined the dependence of the channel duration on temperature and membrane potential and have compared the channel kinetics produced by different agonists and by the neurally released transmitter.

The metathoracic extensor tibiae of adult locusts (*Schistocerca gregaria*) was used⁹ because its junctional glutamate receptors are easily accessible¹⁰, and also because its fibres, being only ~1.5 mm long with a diameter of 100 μ m, have a high input resistance in Cl-free medium (5-8 M Ω) and long space constant (1 cm), which makes them very suitable for voltage-clamp studies. Voltage recording and clamping microelectrodes were inserted within 100 μ m of each other in the middle region of a fibre. L-Glutamate (Na salt pH 8; 0.1 or 1.0 M) or quisqualate (Na salt pH 8; 0.1 M) was applied iontophoretically from a micropipette (50-200 M Ω) positioned between recording and clamping microelectrodes, and at a sufficient distance from the junctional regions to produce a gradual increase in drug concentration at the junction and so reduce effects of fluctuations in ejection rate.

In locust muscle there are at least two types of glutamate-sensitive receptors¹¹. The activation of one type (extra-junctional D and excitatory junctional receptors) leads to membrane depolarisation caused by the opening of channels which are permeable mainly to Na and K ions¹⁰. Activation of the second type (H receptors) produces membrane hyperpolarisation due to the opening of Cl channels. To avoid complications which would result from simultaneous activation of both membrane channels, we abolished the hyperpolarising effect by removing Cl ions from the medium¹¹.

The efficacy of clamping was evaluated from the residual voltage change recorded by a third intracellular microelectrode inserted at various positions along the fibres. Iontophoretic application of glutamate to the muscle membrane produced current fluctuations above the background. The variance of this glutamate noise increased as a linear function of the mean membrane current (μ_i). The energy spectra of the conductance fluctuations follows a $1/f^2$ curve (Fig. 1a and b) which fits well the theoretical relationship

$$S(f) = \frac{\gamma \mu_i (V_m - V_{eq}) / \pi f_c}{1 + (f/f_c)^2} \quad (\text{A}^2 \text{ s}) \quad (1)$$

developed previously for the relaxation of acetylcholine (ACh)-controlled two-state channels in frog endplate⁴ where f = frequency (Hz); f_c = cutoff (half-power) frequency = $1/2\pi\tau$ (where τ is the mean lifetime); γ is the conductance of a single

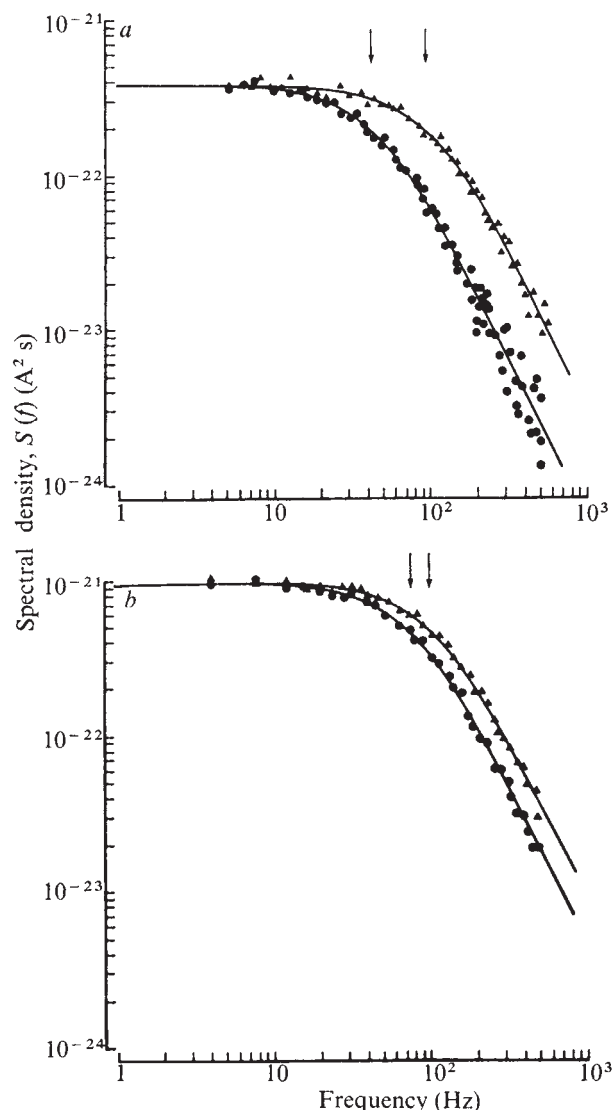


Fig. 1 Energy spectra of glutamate current noise obtained in voltage-clamped muscle fibres. *a*, Temperature dependence of the glutamate noise spectra from a single fibre at $V_m = 104$ mV. $\bullet$, 8.3 °C; $\blacktriangle$, 22.5 °C. The half-power frequencies are: $f_c = 98$ Hz at 22.5 °C and 45 Hz at 8.3 °C, for mean currents of 11 and 9 nA respectively. Vertical axis may be converted to conductances by multiplying ordinate values by 92.46. Single-channel conductances calculated from the fitted curves are $\gamma = 1.03 \times 10^{-10}$ mhos at 22.5 °C and 3.91×10^{-11} mhos at 8.3 °C. For comparison, the curve at 8.3 °C has been scaled by multiplication (simple vertical translation on log-log coordinates) by 1.5. Arrows mark half-power frequencies ($R_{in} = 7.5$ M Ω). *b*, Membrane potential dependence of glutamate noise spectra from a single fibre (different from that shown in *a*) at $T = 23.0$ °C. $\bullet$, 60 mV; $\blacktriangle$, 100 mV. Curves fitted to the data have $f_c = 100$ Hz and $\gamma = 2.54 \times 10^{-10}$ mhos at 100 mV, and $f_c = 76$ Hz and $\gamma = 2.34 \times 10^{-10}$ mhos at 60 mV. $\mu_i = 12$ nA at 100 mV and 16 nA at 60 mV. Vertical axis may be converted to conductances by multiplying ordinate values by $(V_m - V_{eq})^{-2}$. Curve at 100 mV scaled by 0.97 for direct comparison of the cutoff frequencies. Arrows mark half-power frequencies ($R_{in} = 5.0$ M Ω). All spectra shown are from noise records taken during steady local application of glutamate. Data were edited to eliminate spontaneous miniatures. Membrane current was recorded on analog tape (bandwidth 1-2.5 Hz) through an active virtual earth and digitalised (32,768 points at 4,096 Hz sample rate) after low pass filtering (500 Hz, $1/f^8$). The Fourier transform (FFT algorithm) was calculated for 1,024 point segments and averaged after Hann filtering. Spectral values, equally spaced in frequency, are shown on log-log coordinates. No averaging across frequencies was performed. Data were processed with a Linc-8 in double precision floating arithmetic. Control noise spectra (1.25×10^{-23} A² s at 20 Hz) taken immediately before application of glutamate are subtracted point by point to remove background contributions from non-receptor membrane currents and electronics. Clamp quality at the voltage electrode was better than 99.8% at 0 Hz and better than 90% at 1.6 kHz over the entire membrane potential range used. Curves represent the relationship in equation (1).

open ionic channel; V_m = membrane potential and V_{eq} = equilibrium potential for glutamate, taken throughout as zero mV (ref. 11).

Although we positioned the electrodes near a junctional region, the glutamate presumably activated extrajunctional receptors as well. The fact that the noise spectra had a single $1/f^2$ component suggests that the contribution from extrajunctional sites is small, or that the kinetic properties of junctional and extrajunctional channels are very similar. This conclusion is supported by the fact that spectra of current fluctuations obtained during application of glutamate in a bathing solution agreed well with those obtained during iontophoretic application.

As shown in Fig. 1a, decreased temperature prolongs the open state of the glutamate-produced channel from 1.59 ms at 22.5 °C to 3.23 ms at 8.5 °C, giving an apparent single barrier activation energy of $E_A = 8.37$ kcalorie M^{-1} as calculated from the Arrhenius equation. The resulting Q_{10} of 1.45, is lower than that observed for ACh receptor channels⁴. Lowering the temperature has been shown to increase the value of τ derived from glutamate noise in squid nerve cells⁶.

The mean value of the conductance of the single channel opened by glutamate was $\gamma = 2.31 \times 10^{-10}$ mhos at 23 °C, varying in different fibres from 1.0×10^{-10} to 2.6×10^{-10} mhos. In contrast with ACh sensitive channels⁴, the conductance of the single channels opened by glutamate changes with temperature (Fig. 1a). By fitting equation (1) to the measured noise spectra, it was found that γ decreases as the temperature is lowered: in the fibre illustrated in Fig. 1a, $\gamma = 1.03 \times 10^{-10}$ mhos at 22.5 °C and 0.39×10^{-10} mhos at 8.5 °C for values obtained at the same site (t test for difference, $P > 0.01$). This temperature dependence ($Q_{10} = 2.12$) is greater than that of the intra- or extracellular conductivity ($Q_{10} = 1.37$ (ref. 12) and 1.26 (ref. 13), respectively). γ showed no significant correlation with V_m or μ_1 .

Glutamate noise spectra change with membrane potential (Fig. 1b). The duration of an open single channel decreases with hyperpolarisation of the membrane ($\tau = 2.12$ at -50 mV and $\tau = 1.56$ at -110 mV at 23 °C). Figure 2 summarises this relationship for three cells over a 65-mV range of potentials at two temperatures. The voltage dependence of the half-power frequency has a high statistical significance at all temperatures investigated.

Quisqualic acid, a glutamate analogue derived from the seed

Fig. 2 Membrane potential dependence of the cutoff frequency for the energy spectra of glutamate noise. Results shown are from three different cells: ●, 8.0 °C; ▲, 8.5 °C; ○, 23 °C. Spectra were fitted to equation (1) and the cutoff frequencies determined. In terms of the model discussed, the average single-channel lifetime at each V_m and T may be calculated as $1/2\pi f_c$ (s).

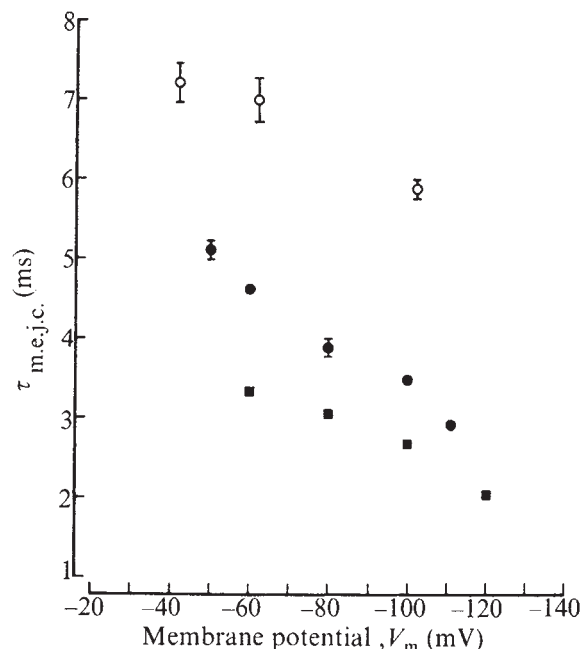
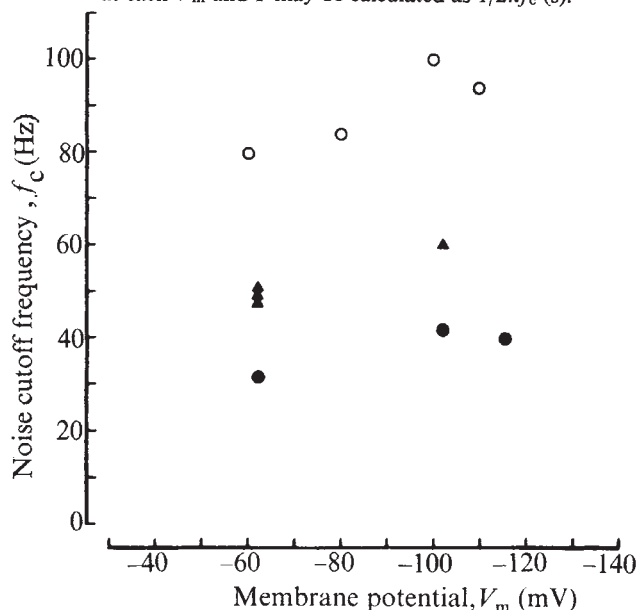


Fig. 3 Membrane potential dependence of the time constant of decay of spontaneous m.e.j.c.s. Results shown are from three different cells: ●, 20 °C; ■, 23 °C; ○ at 9 °C. Each event was digitalised at 5 kHz and the decay phase plotted on log-linear coordinates. The straight line through these points was fitted by the least squares method to give the time constant of the m.e.j.c. Approximately 100 such values were determined at each V_m and T . Histograms of the time constants used in the figure were distributed normally about the mean. The standard deviations ranged from 1.25 ms at 50 mV to 0.38 above 100 mV. t Tests for the confidence of the voltage dependence gave > 0.01 for a 60-mV change and between 0.15 at low V_m and 0.01 at 100 mV for a 20-mV step. The voltage residual during clamping was always less than 5%. Input resistances varied between 1 M Ω at 50 mV and 7.5 M Ω at 100 mV. All values are means $\pm$ s.e. (shown only when greater than the size of the symbols). The larger s.e. obtained at low temperature result from the reduction in γ and the low frequency of m.e.j.c.s.

of *Quisqualis indica*, is several times more effective than glutamate on glutamate receptors of crayfish muscle¹⁴ and rat spinal neurones¹⁵. As the activation of ACh receptors by different drugs produces noise with different characteristics in frog muscle¹⁶, it was of interest to compare the noise resulting from quisqualate and glutamate action in locust.

Iontophoretic application of quisqualate to muscle fibres produced a pronounced increase in μ_1 and in current noise. The energy spectra of the current fluctuations during a steady level of quisqualate action can be described by equation (1) with $f_c = 45$ Hz at 23 °C and -116 mV. In an experiment in which the actions of glutamate and quisqualate were compared at the same sites, the lifetime of the quisqualate-produced channel ($\tau = 3.54$ ms) was 1.8 times that obtained with glutamate. The conductance of the single channels produced by quisqualate ($\gamma = 2.42 \times 10^{-10} \pm 0.25$ mhos, $n = 10$, at 23 °C) and glutamate ($\gamma = 2.32 \times 10^{-10} \pm 0.12$ mhos, $n = 4$, at 23 °C) were, however, almost identical (t test, $P > 0.01$). In addition, quisqualate noise showed membrane potential dependence.

If glutamate is the natural transmitter, and provided the transmitter concentration during a miniature potential subsides very quickly, compared with the life span of an open channel, then the time constant of decay of the spontaneous miniature excitatory junction current (m.e.j.c.), $\tau_{m.e.j.c.}$ should approximate closely to the value of τ derived from glutamate noise (τ_{noise})⁴. Furthermore, both time constants should then display similar voltage and temperature dependence. Figure 3 shows such a relationship for $\tau_{m.e.j.c.}$ which approximately doubles for a 70-mV change in V_m or a 15 °C change in temperature (t test for both, $P > 0.05$).

There are conflicting reports on the voltage dependence of

nerve-evoked excitatory junctional currents at glutamate-sensitive synapses¹⁷⁻¹⁹. Our results are in agreement with those previously reported for the crayfish nerve-muscle junction¹⁸.

Time courses for m.e.j.c.s varied widely in all fibres, a finding confirmed by focal extracellular recording. The mean value of $\tau_{m.e.j.c.}$ estimated from histograms obtained at different voltages and temperatures is longer than the open single-channel lifetime, as derived from the glutamate noise. Nevertheless, a proportion of the m.e.j.c.s, recorded both intra- and extracellularly at each site, gave τ values approximately the same as those of τ_{noise} . This point was checked further by simultaneous recording of focal extracellular miniature potentials and clamped intracellular miniature currents, allowing direct comparison of decay time constants of the same event. Both recordings gave similar τ values. A number of factors could contribute to the prolongation of $\tau_{m.e.j.c.}$, relative to the single-channel duration. These include: barriers to diffusion of transmitter across and out of the cleft; 'compression artefact'²⁰; non-uniform space clamp; synaptic membrane areas with receptors possessing different relaxation times. These possibilities are being investigated.

It can be concluded that glutamate receptors, which mediate depolarisation in locust muscle, open channels with a mean lifetime which depends on temperature, membrane potential and agonist, while the single-channel conductance is dependent on temperature. The increase in conductance of the single channel with increased temperature and the decrease in mean lifetime of the channel with hyperpolarisation, are in marked contrast to the endplate channels opened by ACh. In terms of a dipole model of the glutamate receptor-channel complex, the dipole component parallel to the electric field in the membrane has a polarity opposite to that of the ACh receptor in vertebrate muscle.

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Paradoxical response to amphetamine in developing rats treated with 6-hydroxydopamine

THE clinical syndrome of minimal brain dysfunction (MBD) in children is characterised by hyperactive motor behaviour, decreased attention span, impulsiveness and a variety of cognitive and perceptual problems. A striking

feature of the disorder is the unusual response to stimulant medication. Thus, administration of amphetamine to children with MBD results in a sharp decrease in motor activity. Since the usual pharmacological response to amphetamine is an increase in motor activity, this response has been termed 'paradoxical'. We have described an experimental model in the developing rat that is strikingly similar to the clinical syndrome of MBD found in children². Rat pups treated with 6-hydroxydopamine (6-OHDA) at 5 d of age develop increased motor activity and demonstrate cognitive difficulties in shuttle-box learning between 2 and 4 weeks of age. We now report that administration of (+)-amphetamine reduces the hyperactivity in 6-OHDA-treated rat pups, an effect paralleling the paradoxical response to this agent in children with MBD. The similarity of many of the cardinal features of MBD in an experimental model produced by depletion of brain dopamine in developing rats supports the belief that brain monoamines may be involved in the pathogenesis of this disorder.

All rat pups in a litter were randomly allocated as hyperactive or control animals at 5 d of age. Experimental hyperactivity was effected by intraperitoneal administration of desmethylinipramine (20 mg kg⁻¹) followed after 1 h by intracisternal 6-OHDA (100 µg 6-OHDA·HBr calculated as the free salt in 25 µl sterile saline)²⁻⁵. Control littermates received similar amounts of sterile saline intraperitoneally and intracisternally. Groups of 6-OHDA-treated and control rats were subsequently treated with saline or amphetamine (at doses of 0.25, 0.5, 1.0 and 5.0 mg kg⁻¹) 30 min before monitoring activity and activity was determined for a 60-min interval at 8, 12, 15, 19, 22 and 26 d of age using the following time-sample method^{2,6,7}. Rats were randomised and placed in transparent Plexiglas cages, containing a water bottle and food, which were arranged in three rows on a steel cage rack. At the beginning of each minute the cages were scanned and a written note made of the behaviour of each rat at that time using one of the following categories: inactive—standing or sitting motionless; ambulating—walking or running about the cage; climbing—forepaws on the side of the cage or on water bottle; rearing—both forepaws clear of the floor; eating—gnawing at food; drinking—mouth in contact with water bottle; sniffing—sniffing at any part of the cage or in the air; grooming—any self-washing or licking movements; scratching—scratching body with hind leg. Any cumulative effect of (+)-amphetamine was minimised by the 3-4 d interval between administration of the agent. All measure-

Fig. 1 Effect of amphetamine on activity in control and 6-OHDA-treated rat pups. At 5 d of age animals received intracisternal injections of 25 µl saline or 100 µg 6-OHDA after pretreatment with desmethylinipramine. Activity was determined 30 min after injection of 0.5 mg kg⁻¹ amphetamine throughout the first month of life. Activity is represented as mean $\pm$ s.e.m. First open column, control saline; second open column, control amphetamine; hatched column, 6-OHDA saline; solid column, 6-OHDA amphetamine. *Differs from saline, $P < 0.001$; †differs from saline, $P < 0.05$.

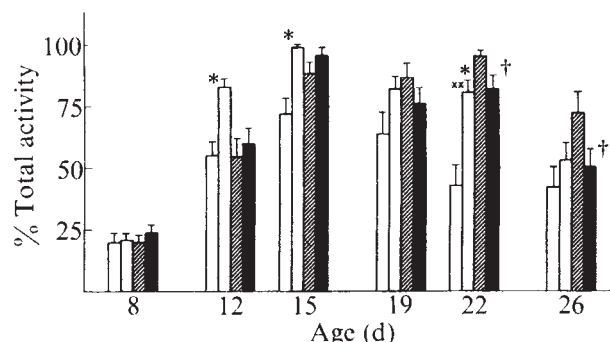


Table 1 Brain catecholamine concentrations

Dose amphetamine (mg kg ⁻¹)	Neonatal treatment			
	Saline	Vehicle	Amphetamine	6-OHDA
				Amphetamine
0.25	(5)	(5)	(5)	(6)
Dopamine	532 ± 25.1		520 ± 36.0	114 ± 43.2*
Noradrenaline	267 ± 11.0		265 ± 20.5	246 ± 8.49
0.5	(6)	(6)	(5)	(6)
Dopamine	488 ± 28.9		480 ± 17.3	191 ± 33.4*
Noradrenaline	345 ± 27.1		278 ± 11.0	313 ± 23.5
1.0	(5)	(6)	(6)	(6)
Dopamine	573 ± 65.2		478 ± 23.5	299 ± 27.6†
Noradrenaline	275 ± 20.6		255 ± 5.6	272 ± 28.3
5.0	(5)	(6)	(5)	(5)
Dopamine	570 ± 16.6		586 ± 37.2	163 ± 64.1‡
Noradrenaline	252 ± 11.6		193 ± 8.23	232 ± 13.0
				143 ± 16.6‡
				209 ± 26.7

*6-OHDA (saline or amphetamine) compared with vehicle (saline and amphetamine) $P < 0.001$; † $P < 0.01$; ‡ $P < 0.05$ (t test).

Dopamine and noradrenaline are means, ng per g wet weight ± s.e.m. 6-OHDA (100 µg) or vehicle administered at 5 d of age. Amphetamine or saline administered at 8, 12, 15, 19, 22 and 26 d of age. Rats killed at 33 d of age. Number of rat pups given in parentheses.

ments were made between 1300 and 1600 to minimise the variation resulting from circadian periodicity. Total activity, ambulation and sniffing activity determinations in each group were subjected to analysis of variance. Rats were killed by decapitation at 33 d of age, their brains were removed rapidly and frozen at -80°C , and analysed within 2 weeks for dopamine and noradrenaline using a fluorimetric assay^{8,9}. At each particular dose 6–8 animals were used in either control or 6-OHDA-treated groups. Differences in catecholamine concentrations between groups were analysed using t test¹⁰.

The intracisternal administration of 6-OHDA to 5-d-old rat pups resulted in significant and comparable reductions in brain dopamine in both amphetamine- and saline-treated animals (Table 1). These reductions in dopamine concentration ranged from 21–39% of control values reflecting the variable amount of 6-OHDA actually reaching dopaminergic terminals by way of the cisternal route, and were in all cases significantly lower than their comparable littermates treated with vehicle solution. This contrasts with noradrenaline concentrations, which ranged from 91–108% of controls and were in no instances significantly reduced compared with the controls.

Total activity was significantly greater in 6-OHDA-treated rats between 2–4 weeks of age ($P < 0.001$) than in littermate controls, confirming results of our previous investigation². This increase in activity was primarily the result of significant increases in both ambulation ($P < 0.001$) and sniffing ($P < 0.001$) that occurred during this period.

Administration of amphetamine at doses of 0.25, 0.5 and 1.0 mg kg⁻¹ to normal developing rat pups resulted in significant increases in activity from 12 d of age, and as early as 8 d in rat pups given 5.0 mg kg⁻¹. This increased activity was due to both increased ambulation and sniffing activity.

In contrast, administration of amphetamine between 2 and 4 weeks of age to 6-OHDA-treated rat pups resulted in a significant reduction in total activity (Fig. 1). This reduction occurred primarily at the expense of ambulatory activity, which was markedly reduced. Sniffing and other stereotyped behaviour did not change. Analysis of variance demonstrated the amphetamine-induced reduction in activity in 6-OHDA-treated rat pups at amphetamine doses of 0.5 mg kg⁻¹ ($F = 5.83$, d.f. 1, 370, $P < 0.05$), 1.0 mg kg⁻¹ ($F = 6.10$, d.f. 1, 359, $P < 0.05$) and 5.0 mg kg⁻¹ ($F = 6.49$, d.f. 1, 360, $P < 0.05$). (+)-Amphetamine at a dose of 0.25 mg kg⁻¹ had no significant effect on activity ($F = 1.4$, d.f. 1, 320,

$P > 0.05$). There was significant correlation between amphetamine dosage and age ($F = 6.38$, d.f. 16, 2,265, $P < 0.001$) as well as between amphetamine dosage and 6-OHDA or saline treatment ($F = 6.35$, d.f. 4, 2,265, $P < 0.001$).

Our data indicate that, in certain conditions, administration of amphetamine results in a decrease in locomotor activity rather than an increase. Such a paradoxical response to amphetamine may be explained most parsimoniously by considering the relationship between amphetamine and brain monoamines. Although their exact nature is unclear, it is generally believed that the stimulant actions of amphetamine are mediated by way of central catecholaminergic mechanisms. Thus, amphetamine is known to increase the amount of dopamine and noradrenaline at the synaptic cleft by increasing granular release and by decreasing neuronal uptake^{11–13}. Controversy exists over whether dopamine, noradrenaline, or both monoamines are responsible for the behavioural effects of amphetamine. The issue is complicated still further by the presence of two well documented dopaminergic systems within the brain. Recent evidence indicates that the mesolimbic dopaminergic pathway is essential for amphetamine-induced locomotor activity, whereas the nigro-neostriatal dopaminergic system seems to mediate amphetamine-induced stereotyped activity^{18–18}.

We suggest that both the hyperactivity and the paradoxical response to amphetamine found in 6-OHDA-treated rat pups may be explained by considering that the nigro-neostriatal dopaminergic system acts as a modulator of excitatory activity. This excitatory activity may be mediated by way of the noradrenergic pathways or by the mesolimbic dopaminergic system. Administration of 6-OHDA affects predominantly the nigro-neostriatal dopaminergic pathway and thus excitatory activity, whether noradrenergic or mesolimbic dopaminergic occurs unrestrained. The denervated receptors in the dopaminergic inhibitory system are, however, supersensitive and so respond to a greater extent to catecholamines released presynaptically by amphetamine than do the undamaged receptors in excitatory pathways, leading to a predominance of inhibitory mechanisms and thus reduced activity.

That such a supersensitivity phenomenon does indeed exist may be demonstrated both behaviourally and pharmacologically. Administration of the dopaminergic stimu-

lating agents apomorphine and L-dopa enhance both the locomotor and stereotypy response to amphetamine in adult rats treated with 6-OHDA (refs 19, 20). Furthermore, recent evidence suggests that the postsynaptic action of dopamine is mediated by way of a cyclic AMP-dependent mechanism. This adenylate cyclase response is significantly increased after denervation of striatal tissue, the presumed pharmacological correlate of supersensitivity²¹⁻²⁴. The parallel between the syndrome produced in developing rats by depletion of brain dopamine and the clinical syndrome, together with the suggestion of reduced turnover of brain dopamine found in a small homogeneous group of children with the disorder²⁵, suggests that brain monoamines may play a role in the pathogenesis of MBD.

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Lesions of ascending dopaminergic pathways decrease forebrain glucose uptake

WE describe the changes in forebrain glucose uptake following various lesions of ascending catecholaminergic pathways. These pathways include dopaminergic fibres arising from the pars compacta of the substantia nigra (A9 of Dahlström and Fuxe¹) and from other cell bodies in the ventral tegmentum (A8, A10) and noradrenergic fibres originating from the locus coeruleus (A6) and from other cell bodies in the midbrain (A7) and medulla (A1, A2, A5)². We have found that destruction of dopaminergic fibres results in decreased forebrain glucose uptake.

Glucose uptake was measured using a new technique developed by Sokoloff *et al.*, which facilitates determination of the rates of glucose uptake of individual structures within the central nervous system (CNS) by using tracer amounts

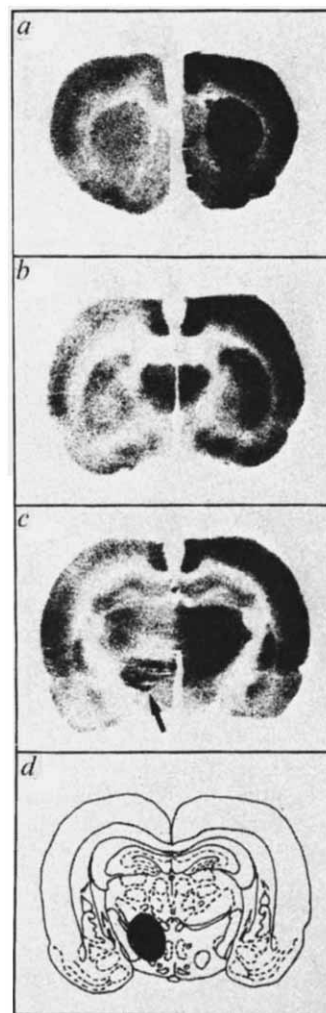


Fig. 1 ¹⁴C-2-deoxy-D-glucose X-ray film autoradiographs of coronal brain sections from an animal with a unilateral electrolytic lesion of the lateral hypothalamus in the region of the ascending catecholaminergic pathways. *d* shows the extent of the lesion, which resulted in ipsilateral depression of forebrain glucose uptake seen at the level of the caudatoputamen (*a*), globus pallidus (*b*), and thalamus (*c*). In *c*, the arrow denotes an area of increased glucose uptake at the rostral end of the lesion, probably representing a region of gliosis.

of ¹⁴C-2-deoxy-D-glucose (2DG)^{3,4}. A pulse of the tracer is injected intravenously and 45 min allowed for completion of the phosphorylation of 2DG to ¹⁴C-2-deoxy-D-glucose-6-phosphate by brain hexokinase. Autoradiographs, made from X-ray film exposed to cut sections of a brain from an injected animal, can be used to calculate the rates of glucose uptake of all the visualised brain structures. The autoradiographs can be used to compare quantitatively the relative rates of glucose uptake of such structures. Thus, the darker the particular area on an autoradiograph, the higher is the glucose uptake^{3,4} in that area.

Recent studies using this technique have mapped qualitatively alterations of regional glucose uptake associated with alterations of functional activity. Regional, activity-related increases in glucose uptake occur in the mammalian CNS during sciatic nerve^{4,5}, vestibular⁶, and olfactory⁷ stimulation; during penicillin-induced seizures⁸; and during periods of swimming⁶. Decreases in glucose uptake follow unilateral eye enucleation and unilateral occlusion of an external auditory canal⁴. Such decreases resulted from elimination of well defined sensory inputs with limited projections. We wished to extend such studies to the metabolic alterations following destruction of intrinsic diffusely-projecting sys-



Fig. 2 Autoradiographs of coronal brain sections from an animal with a unilateral electrolytic lesion in the region of the locus coeruleus. *d* shows the extent of the lesion, which resulted in bilaterally symmetrical forebrain glucose uptake seen at the level of the caudatoputamen (*a*), globus pallidus (*b*), and thalamus (*c*).

tems. To do so, various lesions of the ascending catecholaminergic pathways were made.

A total of 45 Sprague-Dawley albino rats, weighing approximately 150 g each, were anaesthetised with ethyl ether, and either electrolytic or 6-hydroxydopamine (6-OHDA) lesions were placed⁸ stereotaxically in locations described below. For electrolytic lesions, 2 mA of current was passed for 30 s through stainless steel electrodes insulated to within 0.5 mm of their tips. 6-OHDA is believed to cause selective destruction of catecholamine-containing neurones⁹. For chemical lesions, 1 mg of 6-OHDA dihydrobromide (base) and 0.1 mg ascorbic acid were dissolved in 0.5 ml sterile saline and 4 μ l of the solution (8 μ g 6-OHDA) was injected over 4 min using a Hamilton syringe.

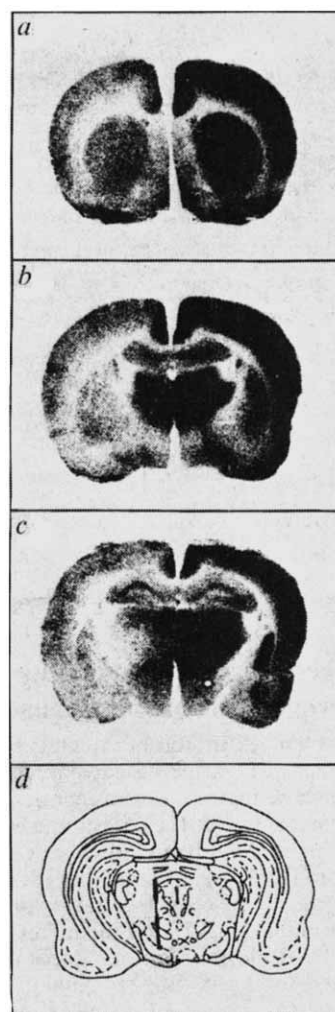
After lesioning, rats were housed in individual cages with food and water *ad libitum* for 72 h. They were then injected intravenously through the tail vein with 50 μ Ci ¹⁴C-2DG (New England Nuclear, 53 Ci mol⁻¹) in 0.25 ml normal saline. After injection, animals were restrained in individual holders for 45 min and then killed with an overdose of sodium pentobarbital. Brains were removed quickly and frozen in isopentane cooled to -70 °C with solid CO₂. Frozen sections (30 μ m) of brain were cut on a cryostat, dried quickly and autoradiographed on X-ray film as described previously¹⁰.

We have made unilateral electrolytic lesions of the lateral hypothalamic area in the region of the ascending catecholaminergic pathways^{2,11}. Nine animals were sub-

jected to such a lesion and 8 had autoradiographs as shown in Fig. 1. The glucose uptake of structures rostral and ipsilateral to the lesion was depressed compared with that of the contralateral side; parts affected were wide ranging and included frontal cerebral cortex, caudatoputamen, parts of thalamus and other subcortical regions. The olfactory bulbs, hippocampus and structures caudal and ipsilateral to the lesion seemed unaffected. Control animals without lesions showed no such asymmetries.

To find out whether the ascending noradrenergic fibres cause this metabolic asymmetry, we made unilateral electrolytic lesions in the region of the locus coeruleus in four animals. These lesions were large (Fig. 2*d*), destroying much of the pons unilaterally at this level, so both the locus coeruleus and ascending noradrenergic fibres from more caudal cell groups (A1, A2, A5) were destroyed. In all these animals, forebrain glucose uptake was bilaterally symmetrical (Fig. 2). Similarly, brains of rats with unilateral (*n*=4) 6-OHDA lesions of locus coeruleus all had bilaterally-symmetrical forebrain glucose uptake. Their autoradiographs were similar to those in Fig. 2. Finally, unilateral electrolytic lesions 1–2 mm further anteriorly (*n*=3), destroying the A7 cell group and all the other

Fig. 3 Autoradiographs of coronal brain sections from an animal with a unilateral 6-OHDA lesion aimed at the substantia nigra. *d* shows the appearance of the cannula track; the lesion resulted in ipsilateral depression of forebrain glucose uptake seen at the level of the caudatoputamen (*a*), globus pallidus (*b*), and thalamus (*c*).



ascending noradrenergic fibres, likewise had symmetrical forebrain glucose uptake.

On the other hand, of 13 animals with unilateral 6-OHDA lesions aimed at the substantia nigra, 4 produced autoradiographs showing a metabolic asymmetry (Fig. 3) similar to that seen after unilateral lateral hypothalamic lesions (Fig. 1). Unilateral electrolytic lesions involving substantia nigra and ventral tegmentum resulted in depressed ipsilateral forebrain glucose uptake in 4 of 6 additional rats, and the autoradiographs were similar to those in Fig. 3.

Our lesions of noradrenergic fibres in midbrain and pons resulted in bilaterally-symmetrical autoradiographs; therefore the ipsilateral depression of forebrain glucose uptake seen after lateral hypothalamic lesions cannot be due to ipsilateral destruction of noradrenergic fibres alone. A forebrain metabolic asymmetry can be reproduced, however, by 6-OHDA lesions around the substantia nigra. Because 6-OHDA can destroy both dopaminergic and noradrenergic fibres⁹, we cannot yet determine whether destruction of ascending dopaminergic fibres alone is sufficient to cause an ipsilateral depression of forebrain glucose uptake or whether simultaneous destruction of both dopaminergic and noradrenergic fibres is required. In either case, we do know that destruction of at least ascending dopaminergic fibres is required.

Our low success rate in animals with 6-OHDA lesions around the substantia nigra (4 of 13) is perhaps due to the relatively large volume occupied by the catecholaminergic elements at this level and so to the difficulty of destroying it all. At the level of the lateral hypothalamus, however, ascending catecholaminergic fibres are gathered together as bundles, and their complete interruption would seem more guaranteed. It is also possible that we destroyed other non-catecholaminergic fibres as they pass through the lateral hypothalamus, and their interruption may have contributed to the high success rate seen there.

The normal level of forebrain glucose uptake in the awake, restrained rat seems to depend, at least, on intact ascending dopaminergic pathways. We speculate that these fibres may modulate forebrain glucose uptake in the intact animal, but we do not know whether this modulation is first mediated through changes in cell firing rates or through the specific dopamine-sensitive adenylate cyclase^{12,13}. Finally, perhaps some of the functions attributed to brain dopamine^{14,15} are somehow related to its control of forebrain metabolic activity.

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Transport of globin mRNA from nucleus into cytoplasm in differentiating embryonic red blood cells

ERYTHROPOIESIS in chick embryos provides a useful experimental system to study specific gene regulation during embryonic differentiation. As they mature, red blood cells become almost totally committed to the synthesis of one set of gene products—haemoglobin peptide chains. Thus, the transcriptional and post-transcriptional processes involved in the expression of the globin genes can be conveniently studied in erythroid cells undergoing maturation.

It is the events that occur in erythroid cells at stages before and during the onset of haemoglobin synthesis that are of the most interest. Very little is known, however, about these early processes, one reason being the lack of an appropriate system whereby precursor erythroid cells at a sufficiently early stage—that is, before haemoglobin synthesis—can be obtained in adequate amounts and purity. This report describes experiments which delineate the initial pattern of transport of a eukaryotic messenger RNA (mRNA) (globin mRNA), from the nucleus into the cytoplasm during cellular differentiation. The results indicate that the synthesis, transport, degradation and translation of globin mRNA in embryonic erythroid cells seem to follow a rigorously regulated and coordinated pattern during embryonic differentiation. Globin mRNA is synthesised and retained exclusively in the nuclei during the initial 24 h of culture. This is followed by an almost complete transfer of globin mRNA from the nuclei into the cytoplasm within the next 24 h. Globin mRNA is thereafter predominantly in the cytoplasm. The relative amounts of globin mRNA in the nuclei and cytoplasm change reciprocally, although the total amount of cellular globin mRNA does not vary significantly during erythroid maturation. Haemoglobin becomes detectable only when 75% of the total cellular globin mRNA is present in the cytoplasm.

Erythroid cells at the erythroblast and later stages of maturation are present in the embryonic circulation of the chick, but the majority of these cells have already begun to synthesise haemoglobin¹. To this end, we have established a culture system in which precursor chick erythroid cells can grow and undergo complete maturation *in vitro*. As a result of the culture conditions and the unique nature of the erythroid cell surface, clean and homogeneous populations of erythroid cells at various stages of maturation can easily be obtained².

In a previous report, we followed the initial synthesis of globin mRNA during maturation of cultured erythroid cells using a very sensitive and specific molecular probe for detecting and quantitating chick globin mRNA—³H-labelled, single-stranded DNA complementary to embryonic chick globin mRNA (globin cDNA)³. The results indicated that significant amounts of globin mRNA were already present in the proerythroblasts by 24 h of culture. These results confirmed the observations obtained using actinomycin D as an inhibitor of RNA synthesis, which showed that haemoglobin synthesis in cultured erythroid cells was no longer sensitive to actinomycin D by 24 h of culture². This separation in the time of the initial synthesis of globin mRNA and the first appearance of haemoglobin was also observed in intact embryos both by direct measurement of globin mRNA using globin cDNA³ as well as indirectly, using actinomycin D (ref. 4).

The observations on the timing of the initial synthesis of globin mRNA during erythroid differentiation led to considerations of post-transcriptional regulation of the expression of globin genes. It is now generally accepted that globin mRNAs are very stable molecules⁵⁻⁷. Very little is known, however, about the events that occur from the

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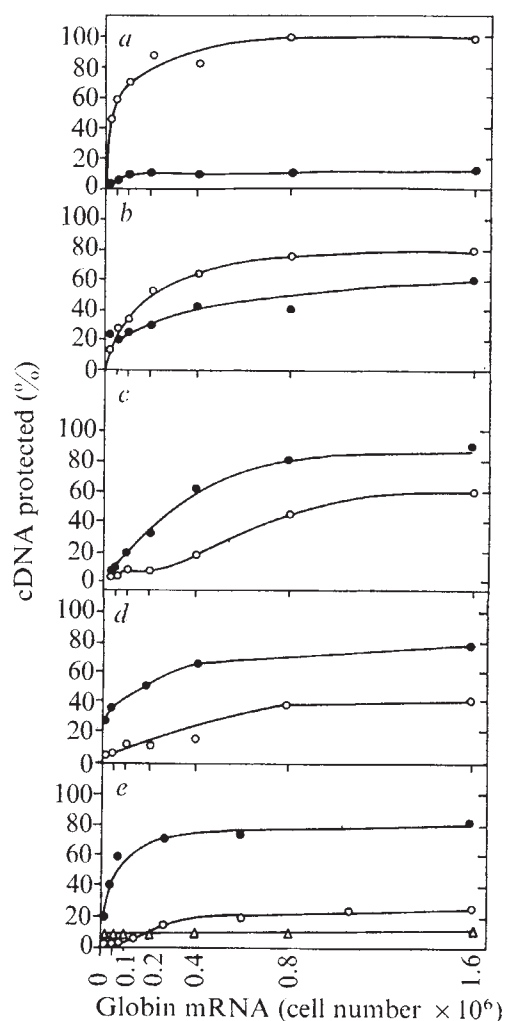


Fig. 1 Hybridisation of 7-d embryonic chick globin cDNA to nuclear and cytoplasmic RNA from erythroid cells at different times of culture. Erythroid cells were collected from the culture medium after various culture periods and washed several times with isotonic saline. Aliquots were taken for cell counts using a haemocytometer counting chamber and for preparation of slides. The slides were fixed in methanol, stained with benzidine reagent for haemoglobin and counterstained with mixed Wright Giemsa. The sensitivity of benzidine is estimated to be in the range of a few picograms haemoglobin per cell¹¹. A total of 200 cells was scored with respect to their reaction to benzidine, and the percentage benzidine-positive cells calculated. The separation of nuclei from cytoplasm was accomplished as follows: the cell pellet was resuspended in 9 volumes buffer containing 0.01 M NaCl, 0.01 M Tris, pH 7.4, 0.003 M MgCl₂, 0.5% NP40 and 100 µg yeast transfer RNA (tRNA) for 2 min. Nuclei were pelleted by centrifugation at 1,400g for 15 min and the supernatant collected. The nuclei were washed once with the same buffer except no yeast tRNA was added. The supernatants were pooled, and 100 µg yeast tRNA was added to the nuclear pellet. RNA was extracted from the nuclear pellet and the supernatant using the phenol-chloroform-isoamyl alcohol technique. The ³H-cDNA input was 300–350 TCA-precipitable c.p.m. per hybridisation reaction. The actual nanogram quantities of chick RNA used per reaction could not be determined because of the presence of yeast tRNA carrier, and therefore was normalised to the number of erythroid cells from which the RNA was extracted. Total cellular RNA extracted from cultured embryonic chick heart fibroblasts was used as control. ○, Nuclear RNA; ●, cytoplasmic RNA; △, total cellular RNA from embryonic chick heart fibroblasts. *a*, 24 h, 0% benzidine+; *b*, 30 h, 0% benzidine+; *c*, 44 h, 2% benzidine+; *d*, 50 h, 15% benzidine+; *e*, 72 h, 60% benzidine+.

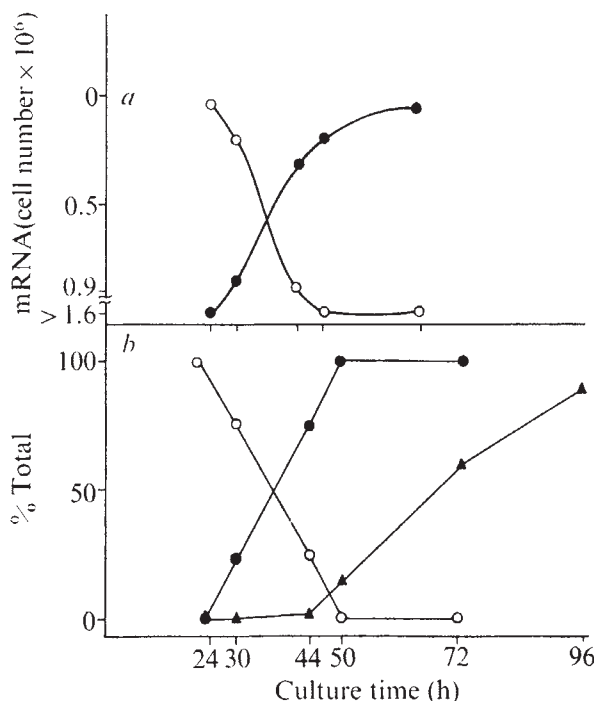
time of the initial synthesis of globin mRNA in the nuclei until the time when they are used for globin synthesis in the cytoplasm. It is reasonable to assume that the mRNA molecules must somehow move from the nuclei into the cytoplasm. But when and in what fashion mRNA transport occurs is hitherto unknown.

The experimental design used globin cDNA as a probe to quantify, as well as establish, the intracellular location of globin mRNA in erythroid cells during differentiation. Erythroid cells were collected after various times of culture, the nuclei were separated from the cytoplasm and the globin mRNA content of each fraction measured using labelled globin cDNA. cDNA was prepared using globin mRNA isolated from 7-d chick embryonic red cells as template³. Molecular hybridisation was carried out using the technique described by Ross *et al.*⁸, except that 5-µl reaction mixtures were used. The amount of labelled cDNA protected by hybridisation with homologous RNA was determined by digestion with *Aspergillus oryzae* S1 nuclease as described previously³.

Figure 1*a–e* shows the results of the hybridisation reactions between globin cDNA and RNAs extracted from the nuclei and cytoplasm of cells after various culture times. The percentage of cDNA protected was plotted against increasing amounts of RNA present in each reaction mixture. Total cellular RNA from cultured chick fibroblasts was used as control (Fig. 1*e*).

The results show that the initial transport of globin mRNA from nuclei into cytoplasm in differentiating erythroid cells follows a well regulated time course. Globin mRNA is synthesised and retained in the nuclei during the initial 24 h of culture, whereas very little, if any, is present in the cytoplasm. A considerable amount of globin mRNA

Fig. 2 *a*, Amounts of globin mRNA in nuclei and cytoplasm of erythroid cells at different times of culture. Ordinate shows amount of globin mRNA needed to give 50% protection of the cDNA in each reaction. Units were normalised to the number of erythroid cells from which the RNA was extracted. ○, Globin mRNA in nuclei; ●, globin mRNA in cytoplasm. *b*, Relative amounts of globin mRNA in nuclei and cytoplasm of erythroid cells and cells containing haemoglobin at different times of culture. ○, Globin mRNA in nuclei; ●, globin mRNA in cytoplasm; ▲, cells containing haemoglobin.



then moves into the cytoplasm during the next 24 h of culture such that, by about 36 h, there are equal amounts of globin mRNA in the nuclei and the cytoplasm. By 44 h and thereafter, globin mRNA is predominantly in the cytoplasm.

From the 50% protection levels of cDNA in the hybridisation reactions, it is possible to estimate the absolute, as well as the relative, amounts of globin mRNA present in the two cellular compartments after different times of culture (Fig. 2a and b). The most striking observation is that the transport of globin mRNA does not follow immediately on synthesis. It is possible that this delay of transport may be due to the processing of mRNA from precursor molecules. The signal for the initiation of transport is unknown. Furthermore, since there is no overall change in the total amount of cellular globin mRNA during the 3-d culture period, this maximum level of globin mRNA is maintained regardless of its intracellular location. Note also that the amount of globin mRNA in the cytoplasm is a direct reciprocal of the amount of globin mRNA in the nuclei. There are several possible explanations to account for this observation: one is that globin mRNA is stable and does not turn over, so that once it is synthesised during the initial 24 h of culture, it remains intact in the cells for the duration of the culture period; a second possibility is that there is no new synthesis nor degradation of globin mRNA during the 24 h when it is being transported into the cytoplasm, but a steady state of synthesis and turnover is established thereafter. Neither of these possibilities seems likely, however, when we consider that the erythroid cells divide approximately once every 10–14 h at this time^{8,9}, but that no dilution of globin mRNA on a per cell basis occurs. A more likely explanation is that new globin mRNA is synthesised during this time, but the rates of synthesis, transport and degradation are coordinated such that the relative amounts of globin mRNA in the nuclei and cytoplasm change reciprocally, but the total amount of cellular globin mRNA remains unchanged. Evidence for this possibility awaits a thorough study of the rates of synthesis as well as rates and site(s) of degradation of globin mRNA in differentiating chick erythroid cells.

With regard to haemoglobin synthesis, the results show that haemoglobin becomes detectable only after 75% or more of total cellular globin mRNA is present in the cytoplasm (Fig. 2b). It is possible, however, that globin synthesis commences immediately after the first molecule of globin mRNA appears in the cytoplasm, but cannot be detected as haemoglobin until some time later. Studies are under way to establish the time when globin mRNA is first used for globin synthesis.

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Effect of ribothymidine in specific eukaryotic tRNAs on their efficiency in *in vitro* protein synthesis

RIBOTHYMINDE (m^5U or rT), the most common methylated nucleoside found in tRNA, is always at the 23rd position from the 3' end in the nucleotide sequence GT ψ CPurine^{1,2}. So far ribothymidine has been found in essentially all prokaryotic tRNAs which function in protein synthesis, but not in several eukaryotic 'elongator' tRNAs^{3–6}. In the case of wheat germ, all the glycine tRNAs, several species of threonine tRNA and one species of tyrosine tRNA have an unmodified uridine in the position usually occupied by ribothymidine (ref. 5 and K.B.M., D. Marcu, and B.S.D., manuscript in preparation). To determine the significance of the absence of rT in these tRNAs, we have methylated them enzymatically with a crude preparation of *E. coli* rT-forming uracil methyltransferase and then compared their efficiency with that of the unmodified tRNAs in a wheat germ cell-free protein synthesising system directed by various natural mRNAs. We report here that these tRNAs function significantly more efficiently in protein synthesis when lacking rT and that the inhibitory effect of rT can be reversed by the polyamine spermine.

The existence of a class of tRNAs containing an unmodified uridine in place of rT is especially significant in view of the evidence suggesting that rT and the tetranucleotide in which it is located are involved directly in the role of a tRNA in protein biosynthesis^{7–16}. Using a crude rT-forming uracil methyltransferase isolated from *E. coli* MRE 600 and S-adenosyl-L-methionine (SAM) as a methyl donor, we methylated *in vitro* the specific rT-lacking tRNAs from wheat germ. Analysis of the methylation reaction showed that rT was the only product, and in the case of pure wheat germ tRNA_{I^{Gly}}, all the rT was found at the 23rd position from the 3' end, as is normal in other tRNAs (ref. 5). About 18% of crude wheat germ tRNAs lack rT and are substrates for methylation: this agrees with the level of rT detected in crude wheat germ tRNA by Littlefield and Dunn¹⁷. Crude wheat germ tRNA was methylated to a level of 0.06 mol of rT produced per mol of crude tRNA, which corresponds with about 30% of the available tRNA substrates. There was essentially no degradation of tRNA during methylation, as shown by the level of aminoacylation of glycine and leucine tRNAs (Table 1) and the absence of an observable change in the elution position of crude wheat germ tRNA and tRNA_{I^{Gly}} on Sephadex G-100 before and after methylation (data not shown).

Initial experiments were performed with a wheat germ cell-free protein synthesising system¹⁸ using tobacco mosaic virions (TMV) RNA, brome mosaic virions (BMV) RNA or chorion polysomal RNA obtained from the chorionating follicular cells of *Antheraea polyphemus* as mRNAs. Analysis of the protein products produced on sodium dodecyl sulphate (SDS)–polyacrylamide gels gave results similar or identical to those reported before for these mRNAs^{19–22}, and confirmed the fidelity of the translation described. But this wheat germ protein-synthesising system could not be used directly to study protein synthetic activity of specific *in vitro* methylated tRNAs because of its high content of endogenous tRNA. We therefore fractionated the wheat germ system and essentially removed the endogenous tRNAs as described before^{23–24}. tRNA was then clearly needed for the incorporation of ³H-leucine and ³H-glycine in the translation of either TMV RNA, BMV RNA or chorion polysomal RNA (Table 2, lines 1 and 2 and Fig. 1a). The extent of protein synthesis with the sham-methylated or untreated crude tRNA controls described

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above was essentially indistinguishable for all mRNAs tested (Table 2, lines 3–6). The use of an equivalent amount of 6% methylated crude tRNA, however, led to a significant reduction in protein synthesis for all mRNAs tested (Table 2, lines 7 and 8). Incorporation of ^3H -leucine decreased by 43% and 36% for TMV and BMV RNAs, respectively, and the amount of ^3H -glycine incorporated with choriion mRNAs decreased by 54% compared with untreated or sham-methylated tRNA.

The time course of protein synthesis directed by TMV RNA was observed in the presence of untreated, sham or

partially methylated wheat germ tRNAs. As Fig. 1b shows, protein synthesis during the first 60 min was inhibited with *in vitro* methylated tRNA compared with untreated or sham-methylated tRNA, and the degree of inhibition depended on the level of *in vitro* methylation.

To study specific tRNAs involved in the inhibitory effect, pure (1.5 nmol per absorbance unit) 100% methylated tRNA₁^{Gly} was mixed with untreated crude wheat germ tRNA and the mixture was tested for protein synthetic activity. With BMV RNA translation, no effect on ^3H -glycine incorporation was observed (Table 2, lines 9 and 10). There was, however, 48% inhibition of choriion mRNA translation (Table 2, lines 11 and 12). Thus, methylated tRNA₁^{Gly} is not involved in the inhibitory effect on BMV RNA translation but inhibits choriion mRNA translation. This was not surprising in view of the very high glycine content of the choriion proteins produced²²: it was the reason we chose this mRNA for study.

Protein synthesis studies were then performed with pre-charged tRNA₁^{Gly} to establish that inhibition is at the level of the ribosome. No appreciable inhibition was observed for

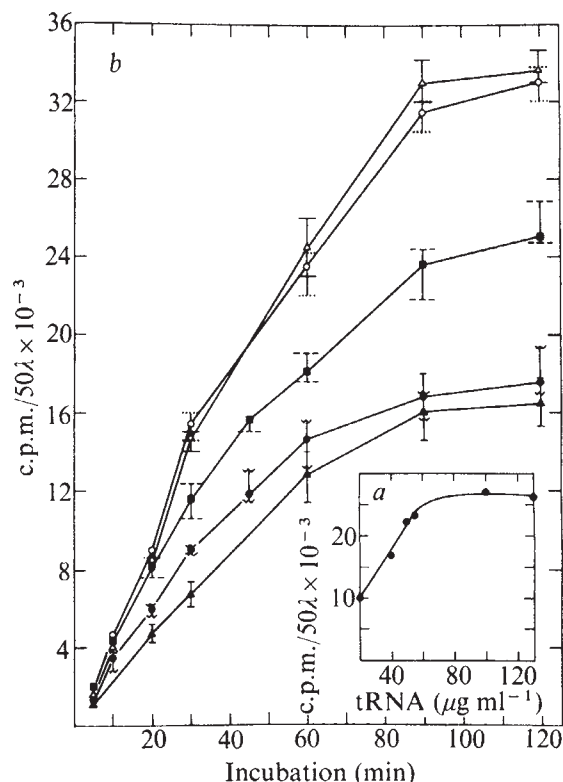


Fig. 1 a, Optimum concentration of crude wheat germ tRNA for the fractionated protein synthesising system. The levels of ^3H -leucine (2 Ci mmol⁻¹) incorporated into protein in a 50- λ protein synthesis assay (with washed ribosomes and an $(\text{NH}_4)_2\text{SO}_4$ -concentrated DEAE-cellulose-treated S100) directed by 4 μg of TMV RNA is shown at various exogenously supplied levels of crude wheat germ tRNA. Reactions were terminated by adding 1 ml of 5% trichloroacetic acid (TCA), heated to 90 °C for 15 min, then cooled and collected on to Whatman GF/A filters which were dried at 150 °C and counted in Omnifluor-toluene as described before¹⁸. Each point represents the average of at least three determinations with a fluctuation not greater than 5%. **b**, Kinetics of protein synthesis directed by TMV RNA with *in vitro* methylated, sham-methylated and untreated crude wheat germ tRNA. All protein synthesis assays were performed with 2.5 μg of the particular tRNA sample in 50 λ . Samples of 5 λ were taken at the times indicated, precipitated, counted as before¹⁸, and corrected for the total incorporation in 50 λ . All five assays were performed in triplicate except for the experiments with non-preincubated tRNA and 6% methylated tRNA, which were performed in duplicate. The 60-min time point for the non-preincubated tRNA sample was obtained in six other experiments with a deviation of not more than 10%. The 60-min time point for the 6% methylated tRNA assay was obtained in three other determinations, also with a fluctuation of not more than 10%. In addition, the 60-min time points for the preincubated, 1.2% and 3.2% methylated tRNAs were reproduced in at least two other assays. The same level of inhibition of methylated tRNA compared with sham or untreated tRNA controls observed at 2.5 μg of tRNA per 50- λ reaction were also observed at a tRNA concentration of 5.0 μg per 50- λ reaction. Proteins were labelled with ^3H -leucine (2 Ci mmol⁻¹). $\circ$, Untreated; Δ , sham-methylated; $\blacksquare$, 1.2% methylated; $\bullet$, 3.2% methylated; $\blacktriangle$, 6% methylated.

Fig. 2 a, Protein synthetic activity directed by TMV RNA in the fractionated cell-free system with crude tRNA (50 $\mu\text{g ml}^{-1}$) at various concentrations of spermine. The reactions were carried out as described in Fig. 1 and ref. 18. Each point was obtained at least in triplicate and the fluctuation was not greater than 12%. **b**, Kinetics of protein synthesis directed by TMV RNA with *in vitro* methylated and sham-methylated crude wheat germ tRNA in the presence of spermine. All protein synthesis assays were performed with 2.5 μg of the particular tRNA sample in 50 λ . Samples of 5 λ were taken at the times indicated, precipitated and counted as before¹⁸, and corrected for the total incorporation in 50 λ . The sham-methylated and 3.2% methylated tRNAs were determined in triplicate and the 1.2% and 6% methylated tRNAs were obtained in duplicate. The 60-min time points of all four assays were redetermined in two separate experiments. Proteins were labelled with ^3H -leucine (2 Ci mmol⁻¹). Symbols as in Fig. 1.

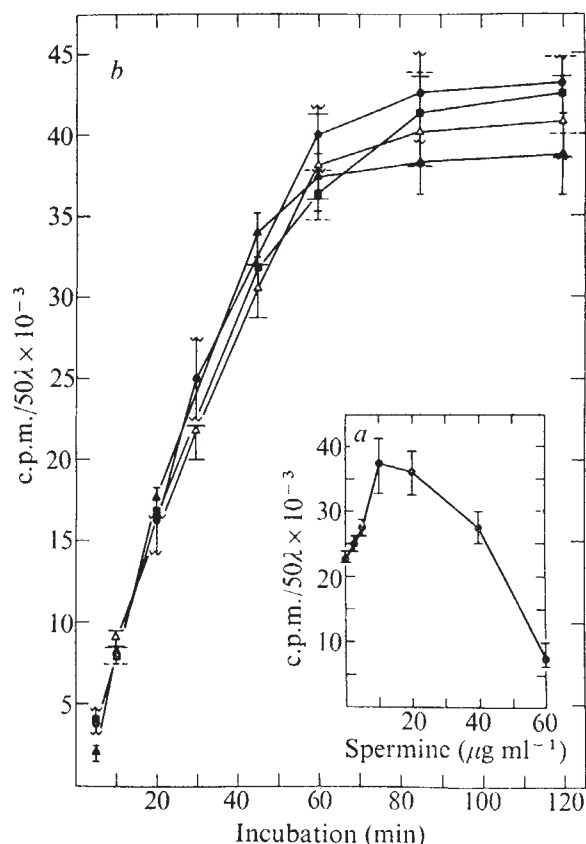


Table 1 Aminoacylation of *in vitro* methylated, sham-methylated and untreated crude wheat germ tRNA

tRNA	³ H-leucine incorporation (c.p.m.)	³ H-glycine incorporation (c.p.m.)
Untreated	2,450	1,040
Sham-methylated	2,400	1,100
1.2% Methylated	2,260	940
3.2% Methylated	2,250	915
6% Methylated	2,200	975

Performed in a final volume of 50 λ containing 50 mM Tris buffer, pH 7.6, 0.5 mM EDTA, 20 mM MgCl₂, 2.5 mM ATP (disodium salt), 10 μ M amino acid (³H-leucine, 2 Ci mmol⁻¹ or ³H-glycine, 1 Ci mmol⁻¹), 2.5 μ g of each tRNA sample and 10 λ of ribosome and tRNA-free wheat germ protein extract. The reaction was incubated at 30 °C for 30 min. Values listed are c.p.m. above a tRNA control performed in duplicate with $\sim$ 10% deviation, and, in the case of methylated tRNAs, they are also corrected for the radioactive methyl label. Sham-methylation refers to the methylation reaction performed in the absence of SAM.

BMV RNA translation (Table 2, lines 13 and 14), which agreed with the comparison of uncharged *in vitro* methylated tRNA₁^{Gly} and uncharged, untreated tRNA₁^{Gly} (Table 2, lines 9 and 10). Chorion mRNA-directed protein synthesis was inhibited using precharged, methylated tRNA₁^{Gly} $\sim$ 48% (Table 2, lines 13 and 14). The degree of inhibition is essentially identical to that observed without precharging the tRNA before protein synthesis (Table 2, lines 11–13), showing that the inhibition does not occur at aminoacylation.

Polyamines stimulate natural mRNA translation in various cell-free systems^{18,25–28}. When an optimum con-

centration of spermine (10 μ g ml⁻¹) was added to the cell-free system (Fig. 2a), the inhibitory effects of methylated tRNA₁^{Gly} on chorion mRNA translation and of methylated bulk tRNA on TMV RNA translation were eliminated (Table 2, lines 15 and 16 and Fig. 2b). The relative protein synthetic activity was increased to the same extent for both sham-methylated and untreated tRNA controls, but the increase was much greater for the methylated tRNA.

The ability of spermine to abolish the inhibitory effect of methylated tRNA may reflect a specific effect of spermine on tRNAs and their function in protein synthesis, perhaps mediated by altering the conformation of the tRNAs. Polyamines bind to ribosomes²⁹ and tRNAs (refs 30–33), and spermine differentially stimulates the translation of specific proteins directed by adenovirus mRNAs so as to reflect the relative levels of the specific proteins made *in vivo*²⁸.

The inhibitory effect on cell-free protein synthesis resulting from *in vitro* rT formation in specific wheat germ tRNAs may explain the absence of rT in these tRNAs *in vivo*. These same tRNAs also lack rT in 14-d-old wheat seedlings (data not shown). rT formation with these specific tRNAs may result in a less active tRNA conformation which is further altered in the presence of spermine to improve tRNA efficiency. The level of spermine varies widely in different organisms²⁹, and the level of spermine in wheat germ is not known and may be insufficient to prevent the inhibitory effects observed. The level of spermine (and perhaps even rT) could vary and constitute an effective regulatory mechanism for protein biosynthesis.

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We thank Drs Barbara Filner for TMV-infected leaves;

Table 2 Protein synthetic "activities" of *in vitro* methylated, sham-methylated and untreated tRNAs in a tRNA-dependent cell-free system directed by natural mRNAs

tRNA	³ H-amino acid	Spermine (10 μ g ml ⁻¹)	TMV inc.* (c.p.m.)	BMV inc.† (c.p.m.)	Chorion inc.‡ (c.p.m.)
(1) None	Gly§	—	—	190	100
(2) None	Leu§	—	200	170	—
(3) Untreated	Leu§	—	22,500	13,500	—
(4) Untreated	Gly§	—	—	1,478	2,570
(5) Sham-methylated	Gly§	—	—	—	2,477
(6) Sham-methylated	Leu§	—	23,000	12,920	—
(7) 6% Methylated crude tRNA	Leu§	—	13,000	8,250	—
(8) 6% Methylated crude tRNA	Gly§	—	—	—	1,150
(9) Untreated tRNA ₁ ^{Gly}	Gly§	—	—	1,396	—
(10) Methylated tRNA ₁ ^{Gly}	Gly§	—	—	1,470	—
(11) Untreated tRNA ₁ ^{Gly}	Gly¶	—	—	—	15,830
(12) Methylated tRNA ₁ ^{Gly}	Gly¶	—	—	—	8,230
(13) ³ H-Gly-tRNA ₁ ^{Gly} **	—	—	—	780	3,020
(14) ³ H-Gly-(methyl) tRNA ₁ ^{Gly} **	—	—	—	600	1,580
(15) Untreated tRNA ₁ ^{Gly}	Gly¶	+	—	—	30,750
(16) Methylated tRNA ₁ ^{Gly}	Gly¶	+	—	—	33,000

Hot TCA-precipitable radioactivity above background (—mRNA control) for 50- λ reactions incubated for 60 min at 30 °C as before¹⁸. All assays contained 2.5 μ g of tRNA unless otherwise noted. Data was obtained at least in duplicate and in some cases in quadruplicate or sextuplicate (TMV data) with an average deviation of 10%. The maximum protein synthetic activity of the fractionated cell-free wheat germ system directed by TMV RNA was approximately 1/8 the level (with and without spermine) observed for the unfractionated extract. This drop in activity was also noted for BMV RNA (1/5) and for chorion polysomal RNA (1/7). The high level of activity of the crude extract was only consistently obtained if the extract was prepared quickly (total preparation time 75–90 min) and stored in liquid N₂ (up to several months) or at –80 °C (up to several weeks). Probably the time required to prepare the fractionated system (10–12 h) resulted in significant loss of protein synthetic activity. This drop in activity was also observed for the crude unfractionated extract after several weeks of storage at –20 °C.

* 4 μ g TMV RNA per 50- λ assay.

† 4 μ g BMV RNA per 50- λ assay.

‡ 10 μ g crude polysomal RNA per 50- λ assay.

§ ³H-glycine, 1 Ci mmol⁻¹; ³H-leucine, 2 Ci mmol⁻¹.

¶ ³H-glycine, 20 Ci mmol⁻¹.

|| 20% of the 2.5 μ g of crude tRNA added was tRNA₁^{Gly} or methylated tRNA₁^{Gly}.

** 2.5 μ g of either methylated or untreated tRNA₁^{Gly} precharged with ³H-glycine (20 Ci mmol⁻¹) + 2.5 μ g of crude untreated tRNA. A standard 50- λ protein synthesis reaction was performed with a 50-fold excess of glycine to prevent deacylation and reacylation of ³H-glycine. Reactions were performed in duplicate with $\sim$ 10% deviation.

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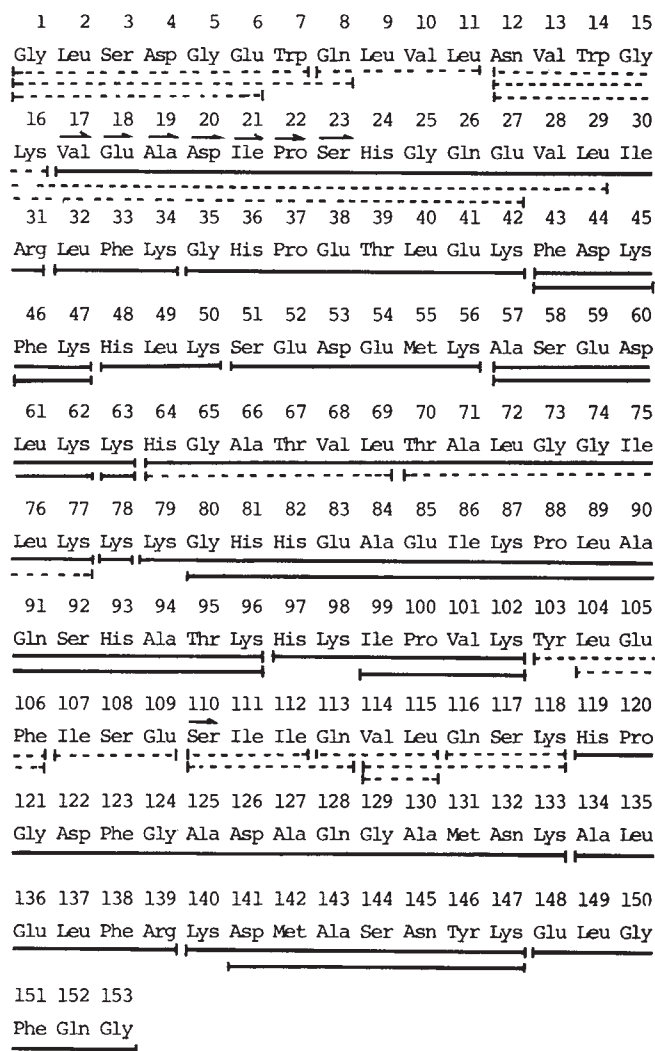


Fig. 1 Orangutan (*Pongo pygmaeus pygmaeus*) myoglobin. Comparison with human myoglobin shows only two differences at positions 23 and 110 where the human residues are Gly and Cys, respectively. Methods used have been described elsewhere¹⁸. —, Tryptic peptides; ----, peptic peptides; —, dansyl-Edman degradation.

Myoglobin of the orangutan as a phylogenetic enigma

The primary structure of the myoglobin of nine anthropoid primates is known from previous work on three ceboids, two cercopithecoids and four hominoids^{1,2}. The most striking result of these investigations has been the nature of residue 110. With the exception of the cercopithecoids and hominoids this position is occupied by alanine in all mammalian myoglobins known, including those of the South American monkeys. In the Old World monkeys, however, residue 110 is serine, and in man and the three apes so far investigated it is a cysteine. It is noteworthy that two of the codons for Ala can change to codons for Ser by a single point mutation, and each of these can change to a codon for Cys by a further single point mutation. There seemed, therefore, to be an evolutionary sequence Ala → Ser → Cys at this position in myoglobin³. In the

α chain of human haemoglobin and that of many other animals the homologous position is also occupied by cysteine⁴. The myoglobin residue at position 23 is also of particular interest among the primates, being serine in the gibbon and the monkeys investigated so far, whereas it is glycine in all other mammalian myoglobins for which the primary structure is known, including those of man, chimpanzee and gorilla.

The amino acid sequence of the myoglobin of the orangutan (Fig. 1) differs from that of man at only two positions, surprisingly 110 and 23. At position 110, orangutan resembles the Old World monkeys by having serine, whereas the other hominoids have cysteine. At position 23 the orangutan resembles the gibbon and monkeys by having serine, rather than glycine as in man, chimpanzee and gorilla. Bearing in mind recent discussion of the phylogeny of catarrhine primates⁵ these findings were unexpected and we have sought to investigate their possible implication.

If the conventional classification of the hominoids is assumed to reflect their phylogenetic relationships, then

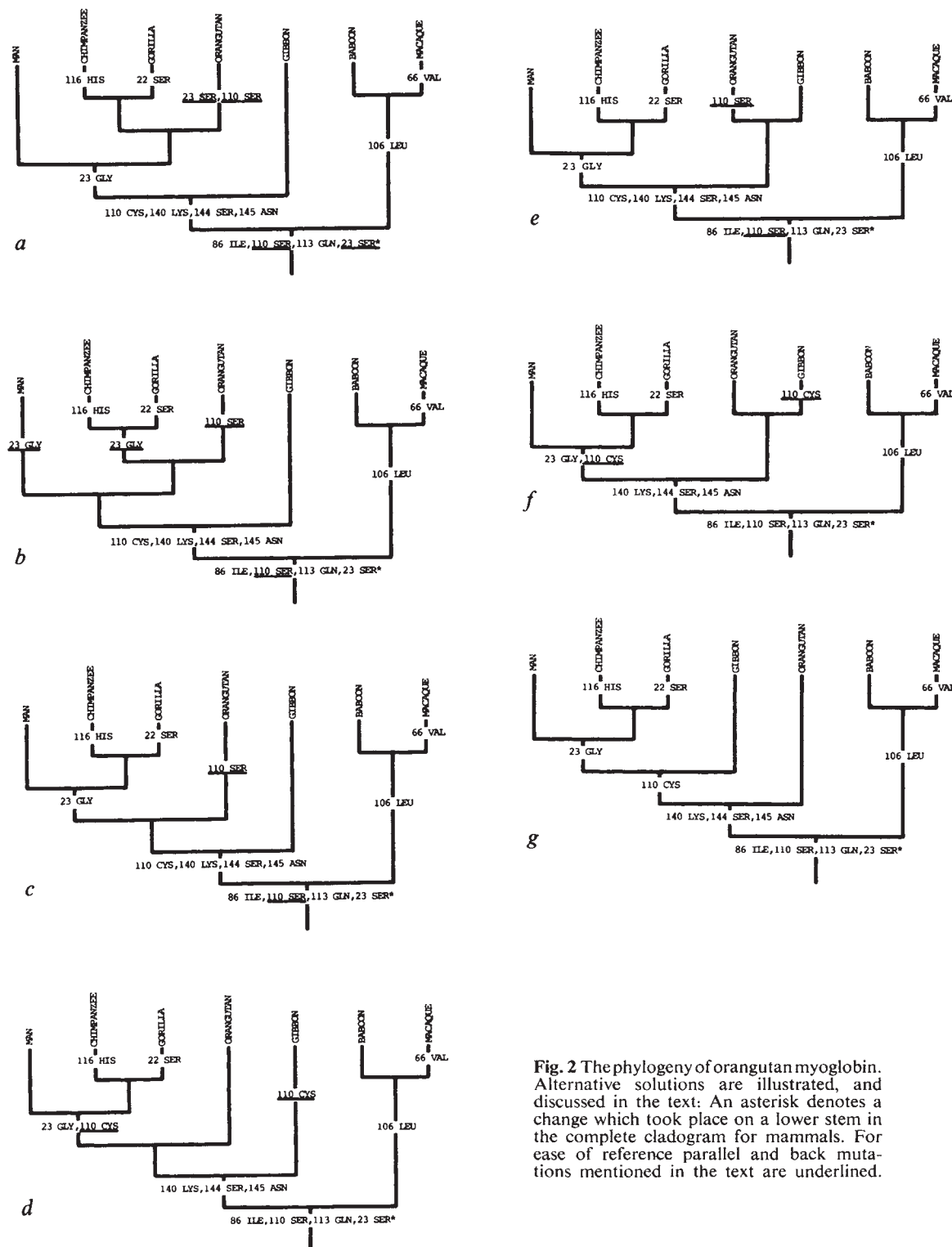


Fig. 2 The phylogeny of orangutan myoglobin. Alternative solutions are illustrated, and discussed in the text: An asterisk denotes a change which took place on a lower stem in the complete cladogram for mammals. For ease of reference parallel and back mutations mentioned in the text are underlined.

the orangutan would share immediate common ancestry with the chimpanzee and gorilla, all being included within the Pongidae, whereas the Homiidae would form a separate lineage leading to man. This pattern of phylogeny is shown in Fig. 2a and b. In both of these cladograms, to account for the observed pattern of residues at the two positions 23 and 110 within the phylogeny of the anthropoids, a total of six single point mutations (hits) is required. In cladogram 2a, 23 Ser and 110 Ser are both shown as back mutations in the line leading to the orangutan, whereas in cladogram 2b the mutations to 23 Gly are shown as

parallel events in the lineage leading to man and in the common stem leading to chimpanzee and gorilla, but 110 Ser remains as a back mutation in the orangutan lineage.

On the other hand, it is generally believed that the family Homiidae originated within the family Pongidae and so it is possible that the orangutan lineage branched from the pongid stem before the divergence of the Homiidae. In this event the chimpanzee and gorilla would share common ancestry with man subsequent to the divergence of the orangutan. This pattern, shown in cladograms 2c and d, can be resolved more economically than 2a and b.

It requires only 5 hits to account for the observed distribution of residues at positions 23 and 110. In cladogram 2c, 110 Ser remains as a back mutation in the orangutan lineage whereas in cladogram 2d 110 Cys is shown as being the product of parallel mutations in the gibbon and in the common stem leading to man, chimpanzee and gorilla.

An equally economical pair of solutions, involving exactly the same series of mutations as those in cladograms 2c and d, can be obtained by linking the orangutan to the gibbon (rather than to man, chimpanzee and gorilla) as shown in cladograms 2e and f. Such a phylogenetic pattern might be taken to imply that there is a fundamental division between African and Asian apes, and that the lineages leading to orangutan and to gibbon separated in Asia rather than in Africa. This is not necessarily so because any hypothesis relating African and Asian apes demands the presence of a viable migration route, and so the divergence between the orangutan and gibbon lineages might have occurred in Africa, along the migration route, or in Asia.

If one seeks the most economical solution to the phylogeny of the residues at positions 23 and 110 then one has to construct cladogram 2g (4 hits), in which the gibbon is so placed that it shares 110 Cys with man, chimpanzee and gorilla, and 23 Ser with the orangutan. This pattern implies that the line leading to the orangutan separated from the hominoid stem before that leading to the gibbon, which is contrary to the current consensus of opinion. Nevertheless, in trying to assess the relative merit of the alternative phylogenetic patterns discussed above, we have taken note of recent statements which seem to indicate that opinion on primate phylogeny is still in a state of flux.

Several years ago, Goodman⁶ and Sarich⁷ cast serious doubts on the phylogenetic pattern represented by cladograms 2a and b. The weight of immunological evidence and the sequences of fibrinopeptides⁸, support patterns 2c and d. Nevertheless, Groves⁹ expressed the view that gibbons were probably descended from the *Dryopithecus* complex, and he regarded their specialisations as being associated with the development of brachiation and reduction in size. Such a view could be accommodated by cladograms 2e and f, and Schultz¹⁰, having reviewed skeletal features within the Hylobatidae, concluded that the siamangs resemble the great apes more closely than do other gibbons, and that this resemblance was particularly to the orangutan. On the other hand, Andrews and Groves¹¹ have little doubt that the gibbons branched off first from the hominoid common stem and their suggestion that *Dryopithecus africanus* is representative of a stage of hominoid evolution when *Pongo* had already branched off the stem but *Homo* and *Pan* had not yet separated, brings us back to cladograms 2c and d.

The only evidence known to us which provides support for the most economical phylogenetic pattern for myoglobin (2g) is given by Darga *et al.*¹², who reported that "with rabbit anti-human albumin serum, gorilla and chimpanzee seem to be almost identical to man while gibbon and siamang show slight divergence and orangutan shows yet more divergence although only about a third as much as Old World monkeys". On the other hand, for rabbit anti-human transferrin, gibbon and siamang diverge more than orangutan, and Darga *et al.*¹² concluded that the weight of immunological and molecular evidence indicated that within the Hominoidea, from the lineage leading eventually to man and the living African apes, an early branch led to the Hylobatidae and a later one to the orangutan.

If we accept the phylogenetic pattern of cladograms 2c and d, then we are forced to accept either a back mutation or a parallel mutation to arrive at the myoglobins of living hominoids. The same number of hits is required for cladograms 2e and f the pattern of which implies that orangutan and gibbon are phylogenetically equidistant from man. This pattern provides the least demanding circum-

stances for resolving the conflicting evidence from albumin and transferrin. If we reject cladogram 2g then we are rejecting the most economical solution. The evidence of other molecules is awaited with interest.

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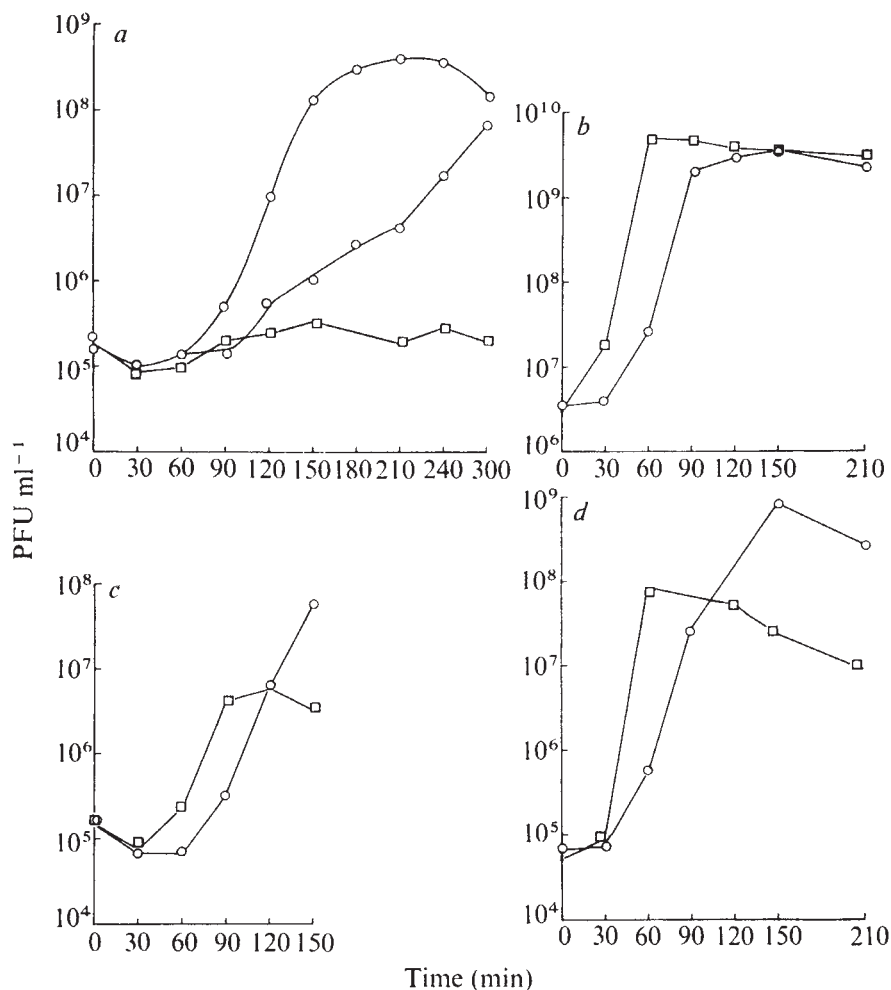
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Host DNA replication or excision repair requirement for ultraviolet induction of bacteriophage λ lysogens

THE mechanism of prophage induction by radiation or chemical agents is unknown, although during the past decade a variety of hypotheses have been advanced¹⁻⁶. Sussman and Ben Zeev⁷ have presented biochemical data that seem to favour the suggestion that DNA intermediates in repair of DNA damage are competitors with the prophage operators for repressor binding. When sufficient repressor is bound to these regions none remains for prophage repression and so induction follows⁷. A consequence of this hypothesis is the prediction that induction

Fig. 1 *a, b* and *c*, Cells were grown to about 10^8 cells ml^{-1} in 10 ml 1% tryptone (Difco), 1% NaCl, 0.2% maltose and 0.01% yeast extract at 34 °C. Cultures were shifted to 42 °C for 1 h and poured into a pre-warmed 600-ml beaker in a 42 °C water bath. At $t = 0$ the culture was irradiated with 254-nm light. All subsequent steps were carried out in subdued light. A sample was then transferred to a flask at 34 °C, and the remaining culture was incubated at 42 °C. At intervals samples were removed, shaken with chloroform and titred for plaque-forming units (PFU) on C600 (*uvr⁺ rec⁺*) by standard techniques. *a*, A second sample was removed from the 42 °C culture at $t = 60$ min and incubated at 34 °C (middle curve). *d*, Cells were grown at 34 °C until $t = 0$, when they were irradiated with the rest of the experimental protocol as in *a, b* and *c*. ○, Incubated at 34 °C; □ incubated at 42 °C. *a*, *dnaA uvrB* λ lysogen irradiated with an incident ultraviolet dose of about 55 J M^{-2} , yielding a cell survival of 0.02%. This dose was found to yield maximum phage induction in control experiments. Cell density at $t = 0$: $9 \times 10^7 \text{ ml}^{-1}$. *b*, *dnaA* λ lysogen. Incident dose 110 J M^{-2} ; cell survival, 3%; cell density at $t = 0$, $1.3 \times 10^8 \text{ ml}^{-1}$. *c*, *uvrB* λ lysogen. Incident dose 55 J M^{-2} ; cell survival, 0.09%; cell density at $t = 0$, $1.6 \times 10^8 \text{ ml}^{-1}$. *d*, *dnaA uvrB* λ lysogen without 42 °C preincubation. Incident dose 55 J M^{-2} ; cell survival, 0.08%; cell density at $t = 0$, $1.8 \times 10^8 \text{ ml}^{-1}$.



should not occur in the absence of these normal repair processes. Here we test and verify this prediction.

Only two repair processes are known to alter the structure of DNA during repair: excision repair, which is absent in *uvrA*, *uvrB* and *uvrC* mutants⁸, and post-replication repair, which seems to be a consequence of the passage of the DNA replication point past a damaged region of DNA^{9,10}.

Both forms of repair were eliminated using a bacteriophage λ lysogen of a *dnaAuvrB* double mutant. The *dnaA* mutation of Abe and Tomizawa¹¹ is temperature sensitive in the initiation of DNA replication, and grows normally at 34 °C. When shifted to 42 °C replication already started continues until complete but no new rounds of replication are initiated. DNA synthesis was undetectable in lysogens of these mutants after 1 h at 42 °C as measured by the incorporation of H^3 -thymidine. DNA synthesis resumed shortly after the lysogens were returned to 34 °C. The *uvrB5* used here was obtained from strain AB2499 of Howard-Flanders⁸.

Lysogens were grown at 34 °C to a density of about 10^8 cells ml^{-1} and shifted to 42 °C for 1 h. They were then irradiated at 42 °C and a sample transferred to 34 °C with the remainder kept at 42 °C. At intervals, samples were removed and assayed for free plaque-forming units.

Figure 1*a* shows that induction does not occur when the irradiated culture is held at 42 °C, whereas it does in the aliquot transferred to 34 °C. When the transfer was delayed until 1 h after irradiation, induction was also delayed (Fig. 1*a*, middle curve). Figure 1*b, c* and *d* shows control experiments; in *b* and *c* the same experimental

protocol was followed with lysogens of single *dnaA* or single *uvrB* mutant hosts. In both cases induction occurs in cultures held at 42 °C as well as at 34 °C, indicating that either excision or replication alone is adequate for induction. In Fig. 1*d* the *dnaAuvrB* lysogen was irradiated before the shift to 42 °C. Induction occurs at 42 °C, an indication that there is no defect in bacteriophage maturation in this double mutant at 42 °C.

Experiments with the radiomimetic antibiotic mitomycin C at $1 \mu\text{g ml}^{-1}$ as the inducing treatment have yielded quantitatively similar results.

As is shown in Fig. 2, little induction was observed over a wide range of ultraviolet doses in non-replicating, non-excising lysogens. The slight inducibility at 5 J m^{-2} has been observed repeatedly and may indicate either residual replication or excision repair, or the presence of alternate, minor pathways of prophage induction.

The absence of ultraviolet induction in non-replicating, non-excising lysogens found here is in disagreement with earlier results of Monk and Gross, who found induction in similar conditions¹². The Monk and Gross experiments differed from these principally in the technical detail of dilution after irradiation. When this dilution step was used with the *Escherichia coli* lysogens of the present study, induction was obtained in cultures held at 42 °C. It is thus possible that dilution somehow initiates replication in a small fraction of the cells, a possibility which was not examined by Monk and Gross.

Although the results reported here can be taken to favour the alternate binding site hypothesis of Sussman and Ben Zeev, they do not rule out the possibility that the

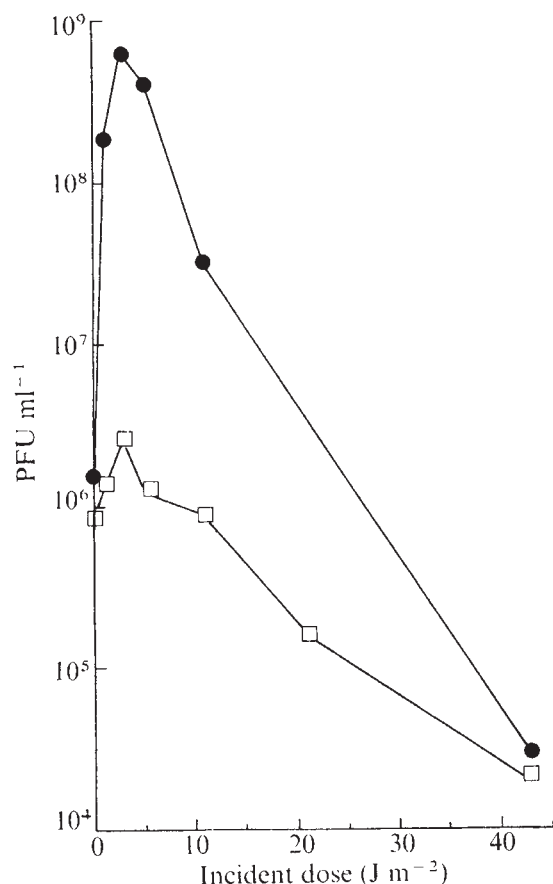


Fig. 2 Dose response of ultraviolet induction: 10 ml *dnAuvrB* mutant was grown to a concentration of about 2×10^8 cells ml⁻¹ at 34 °C in tryptone salt medium described in Fig. 1, collected by centrifugation and resuspended in 20 ml M9 medium¹³ supplemented with 0.5% decolourised casamino acids, 0.01% thiamine and 0.2% glucose. This culture was incubated with shaking at 42 °C for 1 h, and irradiated as described in Fig. 1. At various doses two samples of 1 ml each were removed with a preheated pipette. One sample was incubated at 42 °C, the other at 34 °C for 3 h. Great care was taken to prevent cooling of the samples before the 3-h incubation was over. Chloroform (0.1 ml) was added to each of the incubation tubes, and the tubes shaken at 34 °C for 2 min. The titre of free phage was assayed as before. ●, Incubated at 34 °C. □, incubated at 42 °C.

repair intermediates are also alternative binding sites for repressors of other operons. For example, it is possible that the resulting derepression could induce an 'error-prone' repair system which in turn would cause bacteriophage induction⁶. In view of Sussman's⁷ observations, however, that the λ repressor binds to DNA isolated from ultraviolet-irradiated and mitomycin-treated cells⁷, competition between prophage operators and repair intermediates for λ repressor seems to be the simplest way to account for the observed results.

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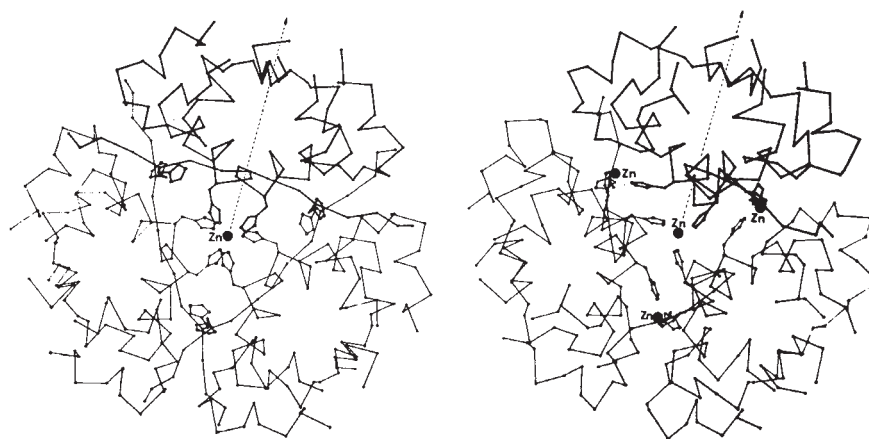
Structure of insulin in 4-zinc insulin

DURING his experiments on slow acting clinical preparations of insulin, Schlichtkrull found that a second form of rhombohedral insulin crystals appeared when the sodium chloride ion concentration in the solution was 6% or more¹. The new crystals contained four zinc ions per hexamer of insulin compared with two in the original crystals. Both forms crystallised in the space group R3 and had similar lattice constants: $a=82.5$ Å, $c=34.0$ Å for 2-zinc insulin, and $a=80.7$ Å, $c=37.6$ Å for 4-zinc insulin crystals². Both, from an early application of the rotation function³, also seemed to be very similar in structure, and to contain in each asymmetric unit, two insulin molecules related to one another by non-crystallographic twofold axes of symmetry. We have now found, from electron density calculations on 4-zinc pig insulin, that the apparent similarity of the crystal structures conceals a surprisingly large change in the conformation of one only of the two insulin molecules in the unit.

The electron density map of 4-zinc pig insulin was phased on data from three isomorphous derivatives; a lead acetate derivative (3 sites) to 2.8-Å resolution; a mercuric acetate-2-hydroxybenzaldehyde derivative (3 sites) to 4.5 Å; and an iodo derivative formed by covalent attachment of iodine to tyrosine (4 sites: one of which proved to be a change in off-axial zinc ion occupancy or temperature factor) to 4.5 Å (ref. 4); measurements on anomalous scattering were made on each crystal. The contributions of the last two derivatives in the phasing calculations were small and the overall figure of merit 0.67. The map is easily interpretable, however, by comparison with that of 2-zinc insulin⁵; over one of the pair of insulin molecules in each unit the electron density is superimposable, over the other marked changes in residue arrangement can be followed. Only a few residues, which are on the protein surface, are not well defined.

In 4-zinc insulin, as in 2-zinc insulin, there are three equivalent dimers associated around the threefold axis to form a hexamer; the two structures are compared in Fig. 1. There are two zinc ions on the threefold axis in 2-zinc insulin, attached through B10 histidine residues to the two very similar molecules, I and II, of each insulin dimer. In 4-zinc insulin, one zinc ion appears on the threefold axis, attached to molecules nearly identical with molecule II of 2-zinc insulin. The remaining zinc ions occupy general sites surrounding the threefold axis, linked by much-changed molecules at position I (Fig. 2). The change is effected by a major rearrangement of the first eight residues of the B chain. These, originally extended, now follow a generally helical course continuing at an angle of about 30° in the direction of the main B9-B19 α -helix.

Fig. 1 A comparison of the 2-zinc insulin and 4-zinc insulin hexamers viewed down the threefold axis. Only α -carbon atoms, histidine side chains and zinc ions are shown. The local twofold axis of the dimers drawn in bold lines is also indicated.

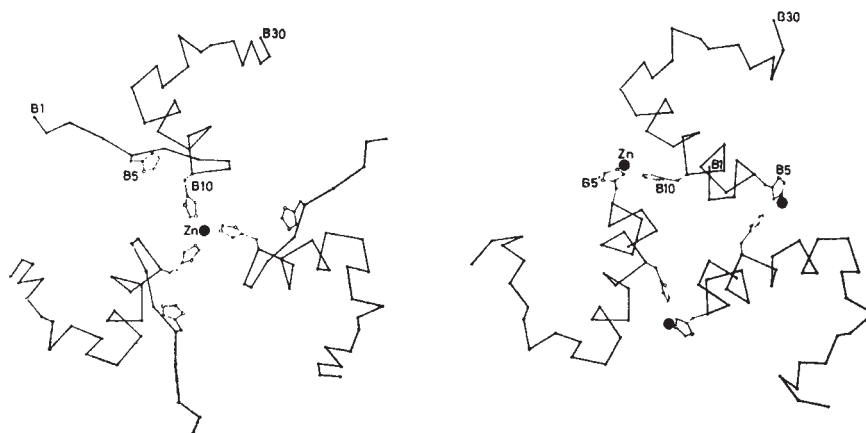


2-ZINC INSULIN

4-ZINC INSULIN

HEXAMER

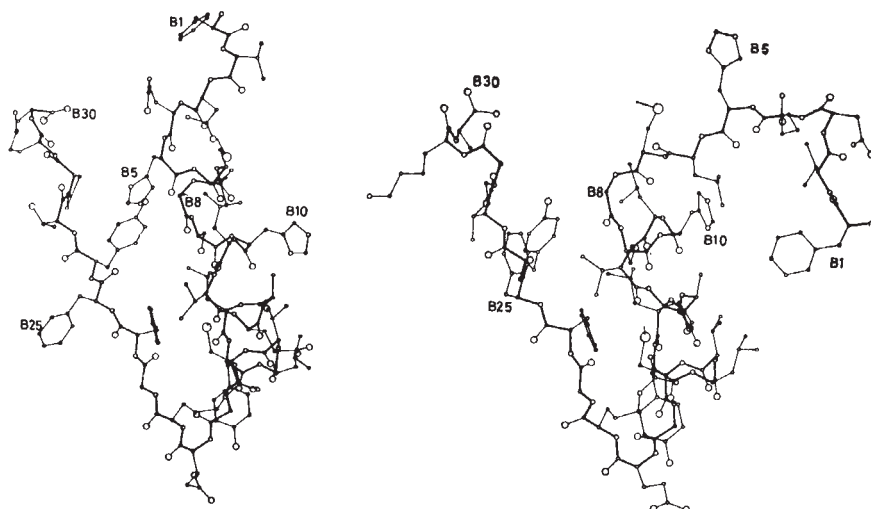
Fig. 2 An identical view to Fig. 1 comparing the molecule I B chains of 2-zinc insulin and 4-zinc insulin, illustrating the changed conformation and zinc coordination.



2-ZINC INSULIN

4-ZINC INSULIN

B Chains Molecule 1



Molecule 1

Molecule 2

Fig. 3 The B chains of molecules I and II viewed from an equivalent direction.

The B5 histidine is moved in the transformations—15 Å across the cell, close to the B10 residue of a neighbouring molecule, which turns towards it. The zinc ion is held between these two histidine residues, tetrahedral co-ordination about it being completed by two water molecules. The change in the B-chain structure pivots about the B8 glycine residue which moves from the 'right-handed' amino acid conformation still present in molecule II, to the helical region of the Ramachandran plot.

The local twofold symmetry which is obeyed quite well in the 2-zinc insulin structure is therefore completely violated by the arrangement of the first eight B-chain residues of insulin molecules in 4-zinc insulin. Between B9 and B30, on the other hand (Fig. 3), the conformations of the two B chains are very similar. Correspondingly, the β -pleated sheet structure and the close non-polar contacts between them are unchanged in this region. The constancy of the structure here confirms our view that these B-chain contacts

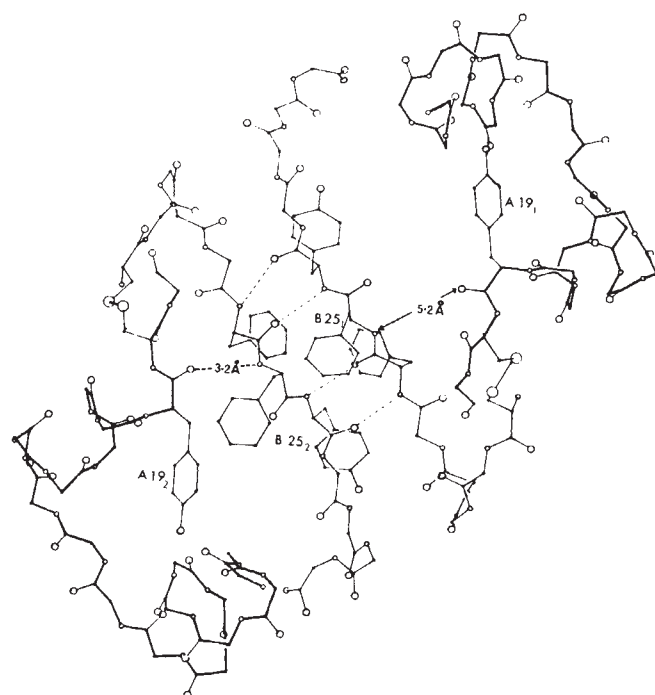


Fig. 4 Part of the 4-zinc insulin dimer viewed along the local twofold axis, showing the appearance of the cleft in molecule I. The region B1-B19 is omitted in both monomers and only the side chains of A19, A20, B19, B24, B25 and B26 are drawn.

define the region of aggregation within the insulin dimer present in solution. The A chains of the two molecules in the 4-zinc insulin dimer are found to be quite similar to one another in conformation, but not in position relative to the B chains. The A chain of molecule I has moved in response to the movement of the A7-B7 disulphide link away from its usual contacts with the B-chain residues. As a consequence, a cleft appears within molecule I (compare Fig. 4). This encourages the asymmetric iodination in the crystal of the tyrosine side chains. The main reaction, which does not occur in 2-zinc insulin, is at tyrosine A19 (two sites with 36% and 26% substitution) and tyrosine B26 (one site with 19% substitution) of molecule I. The enhanced reactivity of molecule I in the insulin dimer is similar to the behaviour of enzyme complexes, such as malate dehydrogenase, in which only half the subunits are active⁸.

The principal change we have observed, the coiling of the initial residues of the B chain, may be encouraged by the sequence in pig and cattle insulin, which is helix-forming in character. Preliminary experiments suggest that ions other than chloride may initiate the transformation, and that the ions enter the crystals. With sodium iodide the crystals formed are similar to, but not exactly isomorphous with, those obtained in sodium chloride solutions; difference electron density maps indicate that the ions enter at different positions on or near the threefold axis. We also have evidence that the 2-zinc crystals themselves can be transformed to 4-zinc insulin crystals simply by adding additional chloride to the mother liquor surrounding them, and that the transformation is reversible (G.A.B., G.G.D. and A. Lewitova, unpublished). The changes are shown by changed lattice constants and by the intensities of diffraction spectra in projection. As might be expected, variable crystal disorder occurs with crystal change; the pathway through the water between the molecules along which the peptide chains must move is clearly tortuous and difficult to follow.

Although the experimental conditions used to effect the transformation to 4-zinc insulin are far from physiological, the phenomena observed are very suggestive in relation to insulin action. 4-zinc cattle insulin displays markedly prolonged hypoglycaemic action and this has led to its use in clinical medicine. Since slow action is less pronounced in 4-zinc pig insulin, it seems that the change must be correlated mainly with different contacts between the molecules which affect solubility and thus insulin release. It is noticeable that the A10 residue, which varies between these species, makes a direct contact with the B5 histidine in the extended helix. The variation of the structure of insulin molecule I, and particularly the opening of the cleft between the A and B chains in the neighbourhood of tyrosine A19, does not seem to impair the overall biological activity of the insulin; evidently it is easily reversible. Many lines of evidence have implicated this region in the action of insulin. Our observations suggest that its flexible character may be one of the important factors in generating biological responses^{7,8}.

We thank the Science Research Council for support, the Wolfson research fund of the Royal Society, the Diabetes Association of Southern California and the US Public Health Service for fellowships to D.A.M., the New Zealand University Grants Committee for a fellowship to G.A.B., and the British Diabetes Association. We also thank Dr J. Schlichtkrull for gifts of insulin.

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Possible dosimeter for ultraviolet radiation

WHILE evaluating the weathering characteristics of the plastics polysulphone and polyphenylene oxide (PPO) we found that they both darkened when exposed to ultraviolet radiation¹. We realised the potential of these polymers as monitors for ultraviolet radiation and are developing them for this use. PPO is now being used to monitor continuously solar ultraviolet radiation at forty sites throughout the world². We are developing polysulphone as a possible personnel dosimeter to measure exposure of the skin to ultraviolet radiation. Two requirements for any ultraviolet dosimeter are: (1) that the wavelength response of the dosimeter should be similar to the erythral action spectrum of human skin, and (2) that the dosimeter should have a monotonic response as the ultraviolet dose increases. The results presented here indicate that polysulphone might well satisfy these criteria.

The polysulphone used (P 1700 Union Carbide) has the structure shown in Fig. 1. It has good stability to thermal³, thermal oxidative⁴ and high energy radiation⁵. It readily undergoes changes in surface properties when exposed outdoors. These changes are accentuated if the material is used as a film, and so this was the form we used.

A high pressure xenon arc lamp was used in conjunction with a single grating monochromator to investigate the response of the polysulphone film to ultraviolet radiation. Irradiance was measured by a thermopile.

When exposed to ultraviolet radiation, polysulphone film was degraded and lost tensile properties. At the same time the initial broad absorption band in the near ultraviolet extended with dose towards the visible. As a measure of the degree of degradation of the polymer, the change in absorbance measured at 330 nm (ΔA_{330}) was noted. This can be done on any standard ultraviolet spectrometer and takes about 10 s.

The change in absorbance was investigated as a function of incident ultraviolet dose for wavelengths ranging from 254 nm to 335 nm. (The film does not respond to wavelengths greater than 340 nm.) At each wavelength, measurements were made for increasing doses until the ΔA_{330} of the film reached approximately 0.3. The values of A_{330} of the unirradiated film were in the range 0.155 to 0.175. Figure 2 shows the results obtained for irradiation at 297 ± 5 nm, that is, at a bandwidth at half-maximum intensity of 10 nm.

For none of the wavelengths studied did the ΔA_{330} increase

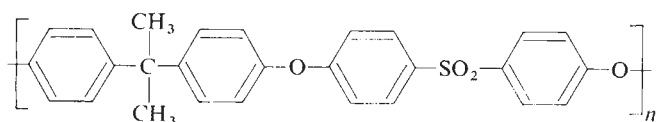


Fig. 1 The structural unit of polysulphone. To prepare the film, the polysulphone was first purified by reprecipitation: the chloroform solution of the polymer was added to a stirred volume of methanol and then the filtered polymer was dried in a vacuum oven at 60 °C. The film was made by spreading a 10% chloroform solution of reprecipitated polymer on a flat glass plate with an adjustable casting blade, producing a film between 36 and 44 μ m thick. The film was removed from the glass plate, dried overnight in a vacuum oven at 60 °C and stored in the dark before use. The choice of film thickness was a compromise between minimising the absorption of wavelengths greater than 330 nm and achieving mechanical strength to facilitate handling. The film was mounted in a standard 5 $\times$ 5 cm photographic transparency holder, constituting the film badge. The badge was usually worn in a matt black holder to eliminate ultraviolet radiation reflected back from the subject.

linearly with dose. The first differential of ΔA_{330} with respect to ultraviolet dose decreased as the dose increased. If, however, the dose-response curves for all wavelengths were normalised at an absorbance of 0.2 to eliminate the different wavelength sensitivities, the mean deviation of each curve from the 297-nm curve was less than 5% at all absorbances up to 0.2. The response of a film exposed to stable sunlight also showed this degree of agreement with the 297-nm curve up to an absorbance of 0.2. At higher absorbances, the agreement lessened; the shorter the wavelength, the less rapid was the increase in absorbance with increase in ultraviolet dose.

This nonlinearity in the response of the film to ultraviolet radiation was not due to oxygen starvation, for a film exposed under high vacuum showed a dose response almost identical to that of one exposed in air, and indicates the photolytic rather than the photo-oxidative nature of the process. The decrease observed in the response of the film at high doses was attributed to the protective filtering effect of the new absorption centres produced. This was supported by the observation that doping unexposed polysulphone film with a known ultraviolet absorbant, 2-hydroxybenzophenone, produced, on exposure to ultraviolet radiation, the same reduction in rate observed in an exposed but undoped sample with the same ΔA_{330} . Investigations with diphenyl sulphone revealed similar radiation sensitivity and build up of filtering products, which suggest that the diphenyl sulphone group in the polymer is responsible for the properties observed.

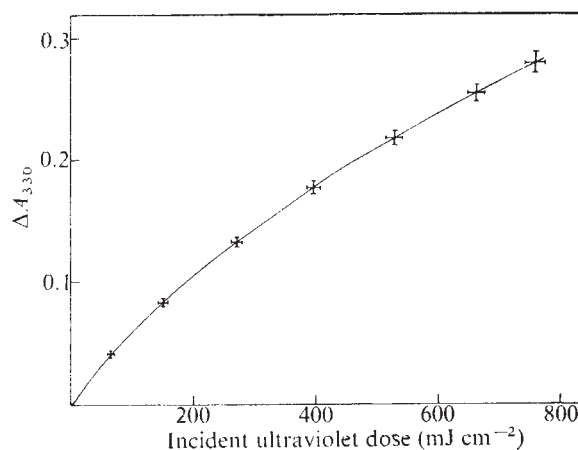


Fig. 2 The variation of ΔA_{330} with incident ultraviolet dose at 297 nm.

When stored, a previously exposed polysulphone film underwent a 'dark reaction', that is, the film exhibited a slight increase in absorbance. After 24 h the ΔA_{330} had increased by about 4%, after 1 week by about 5%. Allowance was made for this factor when necessary.

To derive the wavelength response of the film, the reciprocal of the dose required to produce a ΔA_{330} of 0.1 at each wavelength was plotted as a function of wavelength (Fig. 3). The results were normalised to unity at the most sensitive wavelength. In Fig. 3 the erythral action spectrum of human skin^{6,7} is compared with the action spectrum of polysulphone. Although the agreement is not ideal, it is possible to allow for this, if the relative spectral distribution of the incident radiation is known.

Samples of polysulphone film were irradiated at five levels of irradiance ranging from 0.056 mW cm⁻² to 0.337 mW cm⁻² to give equal doses of 250, 500, 750 and 1000 mJ cm⁻² at a wavelength of 305 ± 5 nm. The resultant values of ΔA_{330} at each dose from the five intensities were within the experimental error of 5% of each other, which indicates that the response of the polysulphone film was independent of dose rate.

For wavelengths less than 315 nm, practically all the radia-

tion is absorbed in a 40- μm film and so a change in the angle of incidence of the radiation will produce negligible changes in response. The refractive index of polysulphone between 315 and 330 nm was 1.63 and at an angle of incidence of 80° the increase in path length was about 25% relative to radiation normally incident on the film. At this angle of incidence, about 20% of the incident radiation was reflected, so the overall effect on the increase in absorbance was minimal.

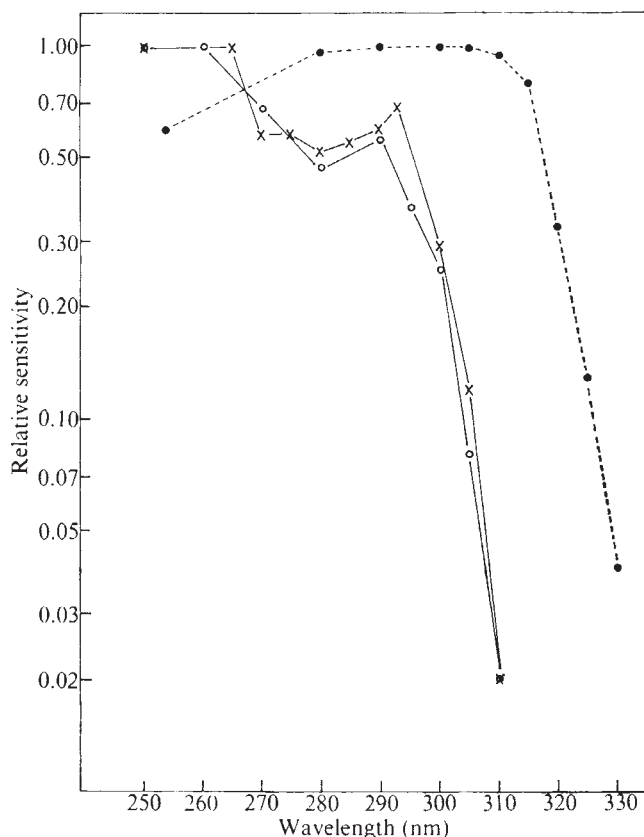
From a calibration curve of the type shown in Fig. 2 it is possible to relate the change in absorbance of a film irradiated by heterogeneous ultraviolet radiation of known relative spectral distribution to an equivalent exposure (F) of 297-nm radiation. The effective erythral dose (D) at 297 nm is then given by

$$D = F \left[\frac{\int I(\lambda) E(\lambda) d\lambda}{\int I(\lambda) G(\lambda) d\lambda} \right] \quad (1)$$

where $I(\lambda)$ is the relative spectral distribution of the incident radiation at wavelength λ , $E(\lambda)$ is the erythral effectiveness at wavelength λ relative to that at 297 nm, assuming the mechanism of response does not change with wavelength and $G(\lambda)$ is the spectral sensitivity of polysulphone in terms of absorbance change, ΔA_{330} , at wavelength λ relative to that at 297 nm, as the biological effects produced by this wavelength have been studied extensively.

The results of this preliminary study of polysulphone as an ultraviolet dosimeter look promising. The method is being used to measure the ultraviolet exposure of bedridden geriatric patients, laboratory personnel working indoors for an 8-h day and hospital gardeners, with a view to estimating the amount of solar radiation needed for the synthesis of vitamin D in the skin. It is hoped that the results will help to quantify previous observations^{8,9}.

Fig. 3 Action spectra of polysulphone and human skin.
●, Polysulphone; —, human skin; ×, ref.6; ○, ref.7.



Although the reproducibility of response of the polysulphone film to a known spectral distribution is of the order of 5%, it is important to know the uncertainty associated with the transformation of polysulphone response to an effective biological dose. This error depends on the nature of the ultraviolet environment and the action spectrum of the biological process under consideration. Careful attention must be paid to the action spectrum of the process to ensure that it declines to zero while the action spectrum of polysulphone is finite. The possibility of making the film thinner and therefore obtaining a better match with the erythema action spectrum is being investigated.

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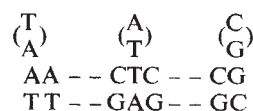
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Erratum

In the article "Similarities in the helical sequences of the repressor-binding sites in the *lac* and λ operators" by M. C. O'Neill (*Nature*, **260**, 550; 1976) the recurring face sequence displayed on page 552 should be



Correction

In the article "Nalidixic acid and bacterial chromosome replication" by G. C. Crumplin and J. T. Smith (*Nature*, **260**, 643; 1976) Figs 2 and 3 have been transposed.

Nature Index and Binders

The **Index** for 1975 is now available, price £2.25. Copies of the 1974 index are still on sale, price £3.00. **Binders** for the journal are also available at £7.00 for four (a year of *Nature* fits into four binders).

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reviews

Slaves to the machine

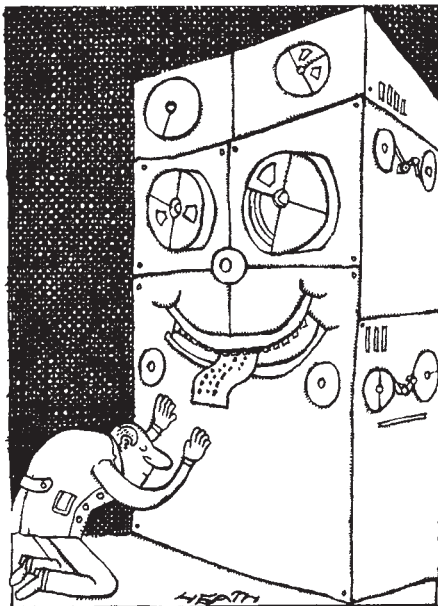
P. N. Johnson-Laird

Computer Power and Human Reason: From Judgment to Calculation. By Joseph Weizenbaum. Pp. xii+300. (Freeman: San Francisco and Reading, January 1976.) \$9.95; £5.95.

No computer centre is complete without one—a gaunt-faced hollow-eyed youth slaving over a hot teletype, working for hours on end long into the night and rarely pausing to satisfy his bodily needs. Should the time available to him on the machine begin to run out, his intensity of work rises to a feverish pitch: he lives dangerously, staking all on some irretrievable alteration of his program. Once off the machine, his life assumes a dull grey quality and is sustained only by sheaves of computer print-outs. He cannot wait to return to where the action is, and will sacrifice all social relations and obligations just to get back on to the system.

Who are these strange dedicated obsessive individuals? They are the new breed of slaves to the machine, the computer 'hackers'. And what are they doing? Almost invariably, they are developing some grandiose general all-purpose super-duper system that once completed will enable other less able programmers to write their own super-duper systems. Weizenbaum, in the most brilliant chapter of his provocative book, has delineated the psychology of computer hackers with a deep and convincing clinical expertise. He shows how the compulsive character of their work relates to a worldview similar to that of their second cousins, the gambling addicts. The computer provides the hacker with a universe, subtle but not malicious, over which he can exercise an absolute and god-like control. The programmer who is corrupted by this power is likely to develop great expertise, but he pays a price. His general all-purpose system is almost always an empty exercise in disembodied programming—that is to say, it fails to make intellectual contact with any other discipline.

The computer hacker is the central metaphor of Weizenbaum's book. He stands for the person who places a single-minded reliance on science as a source of understanding of life, who believes that man is nothing but a machine, and who cheerfully hands over responsibility for making decisions to computers. Weizenbaum's human-



istic scruples are particularly offended by the equation of human thinking with computational processes, and therefore by the new scientific discipline of Artificial Intelligence (AI).

The aim of AI is to construct intelligent machines, and its practitioners are notably devoted to composing computer programs that can perceive the world, or analyse two-dimensional representations of it, and that can conduct conversations in natural language. The first programs capable of rudimentary versions of such skills were written some years ago, but it soon became obvious that it was extremely difficult to test them against human performance. A cynic might say that it was for this reason that the goal of simulation was abandoned in favour of a science of the artificial. Weizenbaum, however, is no cynic; he advances a rather different argument against AI. Since some of his work laid the foundations for the new science, the impact of his book is comparable to that of an anti-vivisectionist tract written by a physiologist whose reputation rests on his operating skills.

What, in essence, is Weizenbaum's case? One can distill three main points from his book. First, certain tasks are too important to be handed over to a computer. It would be immoral as well as dangerous to make computers responsible for certain forms of psychotherapy. Such a proposal, relying on a program in fact devised by Weizenbaum, seems to have been the critical

event that led to the present book. Second, artificial intelligencers suffer from hubris as an occupational disease. They are much given to promising to solve the riddle of the universe . . . tomorrow. "The only reason we have not yet succeeded in simulating every aspect of the real world is that we have been lacking a sufficiently powerful logical calculus. I am working on that problem." So spoke Professor John McCarthy in a perfect specimen of the megalomania that can grip the best of minds at the end of a long day's programming into the night. Weizenbaum, of course, considers that there are certain human propensities, such as the ability to feel desperate or to fall in love, that computers will never be able to display simply because they are not human beings. Third, AI programs are increasingly in danger of exceeding their progenitors' ability to understand them. It is confidently expected that scene analysis programs will soon exceed 1,000K in size, and no-one is likely to be able fully to understand or to communicate their mode of operation.

These are serious criticisms of AI, but even with the worst will in the world they hardly convict the discipline of triviality or immorality. Current computers cannot pick their noses, nor can they fall in love. There is every reason to suppose that they could be devised to carry out the former task, and no reason other than a humanist conscience for supposing that they could not be devised to do the latter. It is a frightening thought—almost as frightening as the idea that our ancestors were apes and that we live on an insignificant planet revolving round an insignificant star. Weizenbaum deserves our gratitude for writing an instructive book that can be read with profit by anyone from a humanist to a computer scientist. It should force us to think more carefully about what we set our computers to do. It should force the artificial intelligencers to reappraise their discipline. No serious practitioner is likely thereby to abandon his science. And the hackers, of course, know that life is nothing but an enormous program devised by the Great Programmer in the sky. □

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Rudiments of physics

Elementary Particles and Symmetries. (Documents on Modern Physics.) By Lewis Ryder. Pp. xxvi+249. (Gordon and Breach: New York, London and Paris, December 1975.) £9.80; £5.00 (International Student Edition).

LEWIS RYDER'S book grew out of lectures given to third-year undergraduates at the University of Kent, and it shows. In my opinion, it is not enough simply to tell students about particle physics. I share what I take to be the author's view that it is preferable, or at least equally important, to teach them how to calculate something—almost anything will do! Any like-minded teacher will find a wealth of material in this admirable book. Of course the topic of symmetries is well suited to this task. The discrete symmetries (time reversal, charge conjugation, parity reversal), the additive conservation laws (charge, strangeness) and the non-abelian groups (isospin, SU_3) are all covered and applied to concrete examples of the strong, weak and electromagnetic interactions. Ten of the thirteen chapters are essentially free of phrases

like: "It can be shown that . . .", which usually herald the onset of the narrative approach. And en route quite a lot of basic information, including some modern developments, is imparted. One surprising omission occurs in the section on the deep inelastic electron-proton scattering data. Having explained why these very important experiments suggest that the nucleon "is like a pomegranate, not like a jelly", I expected, but did not find, some treatment of the naive quark-parton model. Showing how the data suggests that the "seeds" of the pomegranate have one-third integral charges, would have lent further weight to the subsequently raised hot question of particle physics: do quarks actually exist? Towards the end the narrative style inevitably prevails. But even in the last chapter, on the unified theories of weak and electromagnetic interactions, there is enough to show a (keen) beginner how to embark on his own rudimentary model building.

All in all, I think that the book is remarkably successful, and that it caters well for its intended market. The price too is reasonable by current standards, at least for the paperback edition. **D. Bailin**

Molecular spectroscopy

Theory of Molecular Spectroscopy. Vol. 1: Quantum Mechanics and Group Theory of Vibrating and Rotating Molecules. By C. J. H. Schutte. Pp. xvi+512. (North-Holland: Amsterdam and Oxford; American Elsevier: New York, 1976.) Dfl. 170; \$67.95.

THIS is volume 1 of a two volume work and it is not too easy to judge in isolation. It is a strongly mathematical introduction covering vector spaces, matrices, group theory, Hamiltonians in general, both the classical and quantum mechanical form of vibration-rotation interactions, diatomic potential functions and their evaluation, and the interaction of radiation with matter.

The mathematical treatment is thorough and a number of advanced topics are covered, such as the Wilson and Howard Hamiltonian and its modification by Watson, and also the Rydberg-Klein-Rees method of fitting potential curves. The treatment is mostly sound and comparatively free of misprints, and the first impression, strengthened by the early chapter on group theory, is favourable. But I became increasingly unhappy as I delved deeper. It is doubtful whether a standard treatment of the hydrogen atom would be expected in an expensive book on rotating molecules. And as the text gets to real problems, rather than abstract algebra, a lack of authority becomes apparent. Section 1.4 on normal co-ordinates is especially confused; for example, Schutte's use of Cartesians weighted with the square roots of the masses means that the kinetic energy matrix should be the unit matrix, but this is not recognised in this text. And the statement (p241) that the energy "is then $\sim kR^2$, since k is defined as Nm^{-1} " leads to a lack of confidence as does that on p307: "If a molecule does not have a permanent dipole moment, the selection rules for dipole radiation prohibits the occurrence of an infrared spectrum".

There is a need for a comprehensive advanced treatise on the theory of ro-vibration, but part of the difficulty of writing is to choose the important features and give them once only in a modern and consistent form. This text, which has some good sections, tends to give a rather indigestible slab of everything. The price is high and the second companion volume, which will contain the more specific features, is not likely to be less.

D. H. Whiffen

Organic mechanics

Mechanical Design in Organisms. By S. A. Wainwright, W. D. Biggs, J. D. Currey and J. M. Gosline. Pp. xii+425. (Arnold: London, January 1976.) £8.50.

THIS is an ambitious work which brings together much biological information about structural macromolecules and skeletal tissues with the increasing knowledge of their mechanics. It is a book for the specialist and general reader alike, although comparatively few biologists will be sufficiently familiar with materials science, or adequately equipped mathematically, to follow all the engineering phenomena. The first part deals with various tensile, pliant and rigid materials incorporated into skeletal systems. The second part looks into the design of skeletal elements and mechanical support systems, and the third part examines organism-environment interactions. The literature has been assiduously searched and digested, but the book is not an uncritical collection of research results. The authors have rigorously argued their central theme: that structural elements are designed to carry out specific functions in an environment of stresses and strains. Biologists have long appreciated this interplay between form and function, but for many the concept is possibly more an article of faith than a com-

plete understanding. This book provides the comprehension as well as the creeds.

Editorial and technical production reaches a high standard. The book is written throughout in a simple, lucid style (with the occasional touch of humour) making it easy and enjoyable to read. It is elegantly illustrated with numerous drawings and diagrams combining pleasing clarity with meticulous draughtsmanship. I detected few errors, but a curious recurrent mistake is the term 'keratin sulphate' which should be keratan sulphate, or keratosulphate. Naturally there are some points of contention. The dogmatic statement (p105) that α chitin alone exists in Arthropoda is some three years out of date: well-defined examples of β and γ chitin are present in some insect peritrophic membranes and larval cocoons. The '9+2' theory for the structure of α -keratin microfibrils is given another airing, but there is no mention of the 'ring-core' model which fits more closely the X-ray diffraction data and computational studies.

Mechanical Design in Organisms is bound to attract and stimulate considerable interest among biologists and others. It will be an important reference book for some time to come, and as a text it will provide a basis for interdisciplinary teaching of new topics in biophysics and biology degree courses.

W. Kenchington

Desert rats

Rodents in Desert Environments. (Monographiae Biologicae, Vol. 28.) Edited by I. Prakash and P. K. Ghosh. Pp. xvi+624. (Junk: The Hague, 1975.) Dutch Guilders 180.

THIS is one of several recent books devoted to the adaptations of desert organisms. Most have centred around a unifying disciplinary theme, such as comparative physiology, providing integration. This book of 23 invited chapters focuses on a single taxon of organisms sharing a common habitat. Desert rodents, however, are so diverse that appreciating their adaptations requires synthetic interpretation by specialists. Although some of the chapters use a synthetic approach (especially those by Newsome and Corbett; Mares; Rosenzweig, Smigel and Kraft; and Ghosh), many are purely descriptive. As a result the value of the book for the general biologist interested in evolutionary and/or ecological phenomena is diminished; the overall quality would have been greatly enhanced by inclusion of a comprehensive, synthetic chapter based on a thorough reading of the invited chapters, indicating those adaptive patterns which are unique to desert rodents and the advantages they offer.

The primary value of the book is that for the first time (and mainly in a single language: English) there are detailed accounts of the biology of rodents from most of the world's deserts, providing a wealth of comparative information on distributions, general ecology and life histories, activity patterns and population fluctuations, behaviour, competitive interactions, and adaptive physiology. The lengthy chapter on Russian jerboas and gerbils by Naumov and Lobachev is particularly welcome. The chapters vary tremendously in quality and scope with some reporting original research or comprising state-of-the-art messages, whereas others reflect woeful neglect of the current literature or are merely rehashes of ideas and materials previously treated. Lapses in editorial rigour are indicated by numerous typographical errors, and occasional misinformation. Statements of misinformation I found particularly distressing include: "Tropical Dasyurids such as *Antechinomys* . . . do not normally venture into arid habitats", p 394 (the only species of these small Australian Marsupials, *A. spenceri* and *A. laniger* occur exclusively or primarily in arid habitats); "... Heteromyidae . . . the most common rodents in Arizona and the Sahara Desert." p 417 (heteromyids are endemic to the new world).

I found the most interesting paper to be that by Rosenzweig, Smigel and

Kraft, a state-of-the-art message on coexistence and resource allocation in North American desert rodents. As well as summarising what is known, they emphasise equally what is still unknown about the enormous complexity of competitive interaction. Our continuing fascination with desert rodents is well reflected in their statement, "... clearly allocation occurs

. . . But something that is known to occur ought to have an explanation" (p 251).

To the careful reader this book provides a great deal of information on desert rodents, some synthetic explanations, and stated suggestions or unstated gaps where future research could be productively directed.

Richard E. MacMillen

Drug resistance

Acquired Resistance of Microorganisms to Chemotherapeutic Drugs. (Antibiotics and Chemotherapy, Vol. 20.) Edited by F. E. Hahn. Pp. xi+272. (Karger: Basel, London and New York, 1976.) Sfr.139; DM132; \$53.50.

THIS book is a collection of nine chapters by different authors, as follows: Inducible bacterial resistance (Connamacher); Chromosomal mutation to drug resistance in bacteria (Sirotnak); Molecular modifications of anti-microbial agents to overcome drug resistance (Neu); Anti-mutagens and the prevention of chromosomal mutations to drug resistance (Hahn); Genetics of *R* factors (Mitsuhashi *et al.*); Molecular biology of *R* factors (Clowes); Experimental elimination of *R* factors (Hahn); Drug resistance in leukaemia (Hutchinson and Schmid); and Drug resistance in the human malaras (Tiggert and Clyde). There is a preface by Hahn.

Although there are valuable data in the volume, the diversity of authors has produced a very uneven presentation; in parts the English is poor in style and even in grammar; and the same data are presented by different authors, not always with the same lucidity or interpretation.

The price of this book—over £29 at the current rate of exchange—is outrageous. In view of its uneven quality and the many better sources of information, it is not recommended. If information is needed about plasmids and various other genetic aspects of drug resistance, for example, the reader is advised to consult Falkow's *Infectious Multiple Drug Resistance* (Pion: London; distributed by Academic: New York and London, 1975); for review see *Nature*, **258**, 368 (1975). The two books are of about the same size, but Falkow's treatise is greatly superior and costs only £7.70 (\$19.95).

E. S. Anderson

Auger spectroscopy

Photoelectron and Auger Spectroscopy. (Modern Analytical Chemistry.) By Thomas A. Carlson. Pp. xiii+417. (Plenum: New York and London, 1975.) \$39.

THE improvements in electron spectrometers and electron detectors which have been achieved over the past two decades have provided new techniques for the study of the nature of the electron orbitals which hold together the positive framework of matter. The three most profitable areas in which these developments have been applied are ultraviolet photoelectron spectroscopy, X-ray photoelectron spectroscopy and Auger electron spectroscopy. The author of this book, who has had long experience in each of these fields, writes about them in a very lively and authoritative manner.

After the initial chapters which deal with instrumentation and fundamental concepts the subject matter is divided into: photoelectron spectroscopy of outer electron shells; photoelectron spectroscopy of inner shells;

and Auger electron spectroscopy. The first of these topics is mainly concerned with the ultraviolet photoelectron spectroscopy of gaseous molecules and the nature and ordering of the molecular orbitals as revealed by their spectra. The second topic deals largely with the binding energies of the core electrons and how these are shifted by the state of the valence shell environment in which they find themselves—that is, the so-called 'chemical' shift. In the third—the Auger effect—a beam of electrons rather than photons is focussed on the sample, and valence or core electrons removed in an identifiable manner.

Apart from the importance of the basic information on the electronic structure of matter which these effects reveal the fact that they are of low penetrating power makes them extremely valuable for studying surface phenomena such as catalysis, corrosion, adsorption, and so on.

The book is well referenced and contains a great deal of useful tabulated data. It should be valuable both to the general reader and the specialist in the field. W. C. Price

The Encyclopedia of World Regional Geology. Part 1: Western Hemisphere (Including Antarctica and Australia). (Encyclopedia of Earth Sciences, Vol. 8.) Edited by Rhodes W. Fairbridge. Pp. xv+704. (Dowden, Hutchinson and Ross: Stroudsburg, Pennsylvania; Wiley: New York and London; 1975.) \$44.40; £22.30.

THIS is an unusual and successful book written by some 90 authors. Most contribute one or two articles, although the Editor weighs in with a valiant 60 ranging from Antarctica to the Windward Islands. Oceanic islands are done particularly well throughout. It is a true encyclopedia covering the out of the way as effectively as the well known in contributions extending from a column or two on an atoll, to 140 pages on the US. Long contributions are subdivided so any district can be readily picked out, and regional reviews link accounts of individual countries. Ocean floors are not covered: the book sticks to dry land. It will provide basic answers to almost any enquiry requiring knowledge of the geology of a part of the Western Hemisphere. Professor Fairbridge and his colleagues have done a first class job. I look forward to the promised companion volume on the Eastern Hemisphere. **J. Sutton**

Twentieth Century Physics. By Joseph Norwood. Pp. ix+405. (Prentice-Hall: Englewood Cliffs, New Jersey, 1975.) \$16.95.

IN this useful book an attempt is made to present in outline our present understanding of the interactions involving matter. The topics covered are special and general relativity, old quantum theory, wave mechanics and atomic, nuclear and particle physics. On the whole the attempt is successful: the author has striven hard to present arguments which are as simple as possible while at the same time powerful enough to enable results to be derived (or, sometimes, made plausible) rather than merely stated. There is a set of problems at the end of each chapter and this is a particularly valuable feature of the book. Unfortunately, the whole is marred by frequent inaccuracies and an informality of style that sometimes degenerates into sloppiness. For example, Kepler's Laws are wrongly stated, geodesics in space and space-time are confused, it is asserted that terms in $1/C^2$ are small because their value is 10^{-17} (no units being introduced), and so on. In spite of these shortcomings the book will be useful as an auxiliary text for physics undergraduates. **Michael Berry**

Radio and Microwave Spectroscopy. By David J. E. Ingram. Pp. 167. (Butterworth: London and Boston, December 1975.) £4.50.

THIS is very much a book intended for undergraduates in physics or chemistry and must be judged accordingly. This is not easy for someone who knows more about the topics covered—gas microwave rotational spectroscopy, electron resonance and nuclear magnetic resonance. Certainly the language is straightforward, no previous knowledge is expected and the level is appropriate with a text comparatively free of mathematics. The examples are largely taken from Dr Ingram's earlier book, *Spectroscopy at Radio and Microwave Frequencies* (1955), and the diagrams do not give a fair view of modern sophi-

Books brief

stication and one (3.1c) is such a poor photographic reproduction of an oscilloscope screen that it should never have been passed at the galley proof stage. And certainly in 1976 one can no longer maintain, (p109), that for proton chemical shift references "The two protons most commonly used for this purpose are the protons in water . . . and the protons in benzene . . ."; tetramethylsilane is not mentioned. In places, ideas are simplified to a point where explaining more correct views as students advance may be difficult. Thus, zero field parameters in triplet states are referred to as "effective" and then allowed to depend on the direction of the zero amplitude magnetic field (p151). This short book is more appropriate for a weak student in a hurry to get an overview than for more able students. It is also an example of how difficult it is to write a really good elementary text. **D. H. Whiffen**

Graph Theory: An Algorithmic Approach. (Computer Science and Applied Mathematics: A Series of Monographs and Textbooks.) By Nicos Christofides. Pp. xv+400. (Academic: New York and London, October 1975.) £12.50; \$31.00.

THIS book presents a comprehensive and up-to-date account of algorithmic development, which has been going on quietly for over 20 years. The problems of optimisation associated

with graphs are well known, and the lack of mathematical progress in solving them is universally appreciated. What has happened, with the advent of the computer, is the development of some quite powerful and numerically effective methods for solving some of these problems. This book expounds, in a clear style and with ample illustration, the detailed steps of the most important algorithms known at present. The first four chapters are concerned with the theory of graphs, and with the introduction of ideas important in the numerical study of graphs. The chief topics treated are reachability, connectivity, colouring and set covering. There are chapters on the location of centres and medians, on trees, and on shortest paths. Chapters nine and ten are concerned respectively with Eulerian and Hamiltonian circuits, and there is a detailed discussion of some of the classical problems of operational research mathematics, relating to scheduling and the travelling salesman. Chapter eleven deals with flow in networks and the last chapter deals with matchings, transportation and assignment.

No practitioner (whether of operational research or of the social sciences generally) concerned with numerical studies can afford to neglect this book. **L. S. Goddard**

Chromatographic Analysis of the Environment. Edited by Robert L. Grob. Pp. x+734. (Dekker: New York, 1975.) \$49.50.

CHROMATOGRAPHY is undoubtedly a prime method for the analysis of trace components in the environment, and a good book on the subject would be welcomed. The present multi-authored work starts with an introduction to theory and practice, and follows with 18 chapters covering most of the possible permutations of air, earth, water and waste with gas, liquid, paper, thin layer and ion exchange chromatography. One or two of the chapters are very good but too many are narrowly based and simply catalogue methods—many of them rather old. There is a monumental author index of 38 pages and a very poor subject index, which is the reverse of what is required in a book of this kind. This book fails to give the kind of overall picture of environmental analysis required at this stage in the subject and will mainly be useful to those already expert who wish to have comprehensive reference lists before undertaking development of new methods.

John H. Knox

obituary

Grigori Sergeevich Pod'yapol'skii, the Soviet geophysicist and human rights campaigner, died on March 8, 1976.

Pod'yapol'skii was born on October 22, 1926, in Tashkent. In 1949, he graduated from the Moscow Petroleum Institute, and from then until 1953 he worked as a geophysical engineer, doing field work in various regions of the Soviet Union (Ukraine, Siberia, Central Asia, the Arctic). In 1953, he began research work at the Institute of Earth Physics.

During the next seventeen years Pod'yapol'skii was mainly concerned with the theory of earthquakes (in particular the statistics of their occurrence and the propagation of shockwaves). In 1969, he spoke at the International Conference on Tsunamis, in Honolulu on

the relationship between tsunamis and the earthquakes which trigger them.

Pod'yapol'skii's presence in Honolulu was, in retrospect, somewhat surprising. The composition of Soviet delegations to international conferences is decided more in accordance with the political rather than the academic soundness of the would-be participants. By 1969, Pod'yapol'skii was already in official disfavour as a leading member of the growing movement of dissent within the Soviet Union; he was a founder of the Initiative Group for the Defence of Civil Rights in the USSR and a close associate of Academician Andrei Sakharov. His attendance at the Honolulu conference can only be explained, it would appear, by a desire on the part of the Soviet authorities to

make a good showing in the international arena, and as an implicit acknowledgement of the high value they placed on his work. This value, however, they would not acknowledge at home. In the same year, 1969, Pod'yapol'skii was prevented from defending his thesis, and the following year he was dismissed from the Institute on the pretext of "staff cuts", so that his scientific career was effectively brought to a halt.

According to his fellow dissidents, Pod'yapol'skii was a man of great personal charm, and skilled in reconciling the incipient disputes inevitable among those eager to exercise freedom of speech and opinion. He was also a poet; his work reflecting his scientific and humanitarian interests.

announcements

Appointments

Dr **K. G. Binmore** to the Chair of Mathematics at the London School of Economics.

Professor **Gabriele Veneziano** to the Amos De-Shalit Chair in Theoretical Physics at the Weizman Institute.

Professor **Gerald Holton** as the Mallinckrodt Professor of Physics and Professor of the History of Science at Harvard University.

Dr **J. D. Hodge** to the Chair of Botany at Royal Holloway College.

Awards

The **Lenin Prizes** for 1976 were awarded as follows:

to Agasi N. Charakhch'yan, Galina A. Bazilevskaya, Yurii I. Stozhkov, and Taisiya N. Charakhch'yan for work on cosmic ray bursts from the Sun and the solar modulation of galactic cosmic rays;

to Nikolai N. Krasovskii, Aleksandr B. Kurzhanskii, Yurii S. Osipov, and Andrei I. Subbotin for work on the mathematical theory of control systems; to Nikolai N. Semenov for work on the kinetics of complex chemical reactions; to Vladimir S. Sobolev, Nikolai L. Dobrentsov, Vladimir V. Reverdatto, and Vladimir V. Khlestov for work on geological metamorphism;

to Aleksandr S. Spirin, Georgii P. Georgiev, Olga P. Samarina, Murat A. Aitkhozhin, Nadezhda V. Belitsina, and Lev P. Ovchinnikov for the discovery

and study of informosomes;

to Sergei V. Anichkov and Vasilii V. Zakusov for work on the effect on synapses of physiologically active substances;

to Vladimir I. Burakovskii, Leonid A. Bokeriya, and Vitalii A. Bukharin for work on hyperbaric oxygenation in heart surgery;

to Feliks G. Arzhanov, Valeri I. Graifer, Valentin V. Karibskii, Aleksandr V. Sinel'nik, Teygat S. Gainutdinov, and Valentin D. Shashin (Minister of the Petroleum Industry) for work on the automation of petroleum production;

and to Vasilii T. Babenko, Anatolii I. Manokhin, Tamerlan S. Shishkhanov, Nikolai P. Lyazhishev, Nikolai P. Slotvinskii-Sidak, and Aleksandr N. Morozov for a new method of producing vanadium.

The National Academy of Engineering Founders Award to Professor **Mason Benedict** for work on nuclear energy.

The NAE Vladimir K. Zworykin Award to Dr **C. K. N. Patel** for contributions to laser technology.

The Marconi International Fellowship to Dr **H. Inose** for work on computers. The Royal Society has elected Professor **S. Benzer**, Professor **W. H. Munk**, Professor **R. W. Sperry** and Professor **C. H. Townes** Foreign Members of the Society.

The Rank Prize for Nutrition to **Norman W. Pirie** for work on the extraction of protein food from herbage.

The Charles Frederick Chandler Medal to Professor **Kai Seigbahn** for developing ESCA.

International meetings

May 18–21, **Drug Receptor and Drug-Enzyme Interactions**, Namur, Belgium (Services des Relations Publiques, Facultés Notre-Dame de la Paix, 61, rue de Bruxelles, B-5000 Namur, Belgium).

May 20, **Onchocerciasis**, London (Betty Harrison, Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London W1N 4EY).

May 27–28, **Oncogenic Transformation**, Lake Placid (Course Secretary, W. Alton Jones Cell Science Center, Old Barn Road, Lake Placid, New York 12946).

July 5–7, **Theoretical Methods in Polymer Physics**, Leeds (Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

July 6–8, **Breeding Endangered Species in Captivity**, London Zoo (Dr Michael Brambell, The Zoological Society of London, Regents Park, London NW1 4RY, UK).

July 8–9, **Nutrition and Growth**, Cambridge (Dr J. D. Sutton, Hon. Programmes Secretary, National Institute for Research in Dairying, Shinfield, Reading RG2 9AT, UK).

July 12–16, **Properties of Liquid Metals**, Bristol (Meeting Officer, The Institute of Physics, 47 Belgrave Square, London

SW1X 8QX, UK). (Revised date).

July 12-16, **Progress in Marine Research in the Caribbean and Adjacent Regions**, Caracas (Dr H. B. Stewart, Chairman CICAR-II Steering Committee, NOAA Atlantic Oceanographic and Meteorological Labs, 15 Rickenbacker Causeway, Virginia Key, Miami, Florida 33149).

July 12-16, **Catalysis**, London (Sixth International Conference on Catalysis, Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN, UK).

July 13-16, **Psychoneuroendocrinology**, Strasbourg (Professor Paul Mandel, CNRS, Centre de Neurochimie, 11 Rue Humann, 67085 Strasbourg-Cedex, France).

July 27-31, **Transcultural Psychiatry**, Bradford (Conference Secretary, International Congress on Transcultural Psychiatry, Room N2, University of Bradford, Bradford BD7 1DP, UK).

August 4-6, **Applications of X-Ray Analysis**, Denver (Ms Mildred Cain, Denver Research Institute, University of Denver, Denver, Colorado 80210).

August 22-28, **Photosynthetic Prokaryotes**, Dundee (Dr G. A. Codd, Department of Biological Sciences, University of Dundee, Dundee DD1 4HN, UK).

September 3-7, **Annual Convention of the American Psychological Association**, Washington DC (APA Public Information Office, 1200 Seventeenth St, NW, Washington DC 20036).

September 7-9, **Wind Energy Systems**, Cambridge (Organising Secretary, WES, BHRA Fluid Engineering, Cranfield, Bedford MK43 0AJ, UK).

September 28-30, **Cellular Aspects of Neoplasia**, London (Dr G. Hodges, Imperial Cancer Fund, Lincoln's Inn Fields, London WC2A 3PX, UK).

March 30-April 1, 1977, **Mixing**, Cambridge (Deadline for submission of synopses: June 30) (Organising Secretary, 2nd Mixing Conference, BHRA Fluid Engineering, Cranfield, Bedford MK43 0AJ, UK).

July 4-8, 1977, **Rhythmic Activity of Fish**, Stirling (J. E. Thorpe, Freshwater Fisheries Laboratory, Pitlochry, Perthshire PH16 5LB, UK).

July 18-23, 1977, **Physiological Sciences**, Paris (Pr J. Scherrer, Secrétariat du XXVIIe Congrès International des Sciences Physiologiques, UER Pitié-Salpêtrière, Cedex 1300, F-75300 Paris-Brune, France).

August 10-19, 1977, **History of Science**, Edinburgh (Date for preliminary registration: June 30) (Dr Eric Forbes, Congress Secretary, XVth International Congress of the History of Science, The Royal Society of Edinburgh, 22 George St, Edinburgh EH2 2PQ, UK).

Person to Person

As a tribute to **Rene Moricard** a prize of 125,000 B.F. will be awarded for experimental work relating to the initial stages of cancer of the uterine cervix. Manuscripts should be submitted by December 1, 1976 to Dr R. Vokaer, Clinique Obstétricale et Gynécologique, Hôpital Universitaire Brugmann, Place Van Gehuchten, 4, 1020 Brussels, Belgium.

Four bedroom contemporary house in Lexington, Mass. (6 miles from Cambridge), with use of community swimming pool and car included, offered Jan.-July 1977, in exchange for similar or smaller house in Oxford. G. Wolf, 56-235 M.I.T., Cambridge, Mass. 02139, USA.

If you receive or produce a newsletter on biological research, please write giving details of title, publisher, frequency of publication, subject content and subscription rate to Trevor Crane, COBI Research Officer, c/o Prof. J. B. Jepson, Middlesex Hospital Medical School, London W1P 7PN. This information is being sought for the Committee of Biological Information (COBI) so that a list can be compiled. The list will aid research workers in contacting others with similar interests, provide access to another source of information and possibly indicate topics for current awareness bulletins (macroprofiles).

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

September 5-10, 1977, **Social Insects**, Wageningen (Dr J. Beetsma, International Agricultural Centre, Lawickse Allee 11, PO Box 88, Wageningen, The Netherlands).

October 3-7, 1977, **Small-Angle Scattering of X-Rays and Neutrons**, Gatlinburg (Dr R. W. Hendricks, Metals and Ceramics Division, Oak Ridge, National Laboratory, PO Box X, Oak Ridge, Tennessee 37830).

Reports and Publications

Other countries

New Zealand: Ministry of Transport. New Zealand Meteorological Service. Meteorological Observations for 1974—Stations in New Zealand and Outlying Islands, Including the Cook Group, Tokelau Islands, Niue Island and Western Samoa. Pp. 104. \$1.50. Rainfall Observations for 1974—Stations in New Zealand and Outlying Islands, Including the Cook

Group, Tokelau Islands, Niue Island and Western Samoa. Pp. 73 \$1 (Wellington: NZ Meteorological Service, 1974.) [261]

CERN—European Organization for Nuclear Research. CERN 75-18: Radiation Damage to Electronic Components. By S. Battisti, R. Bossart, H. Schonbacher and M. Van de Voorde. Pp. 29. (Geneva: CERN, 1975.) [261]

United States Department of the Interior: Geological Survey, Bulletin 1394-H: The Whitehorn Granodiorite Of the Arkansas Valley in Central Colorado. By Chester T. Wucke. Pp. iii+8. (Washington, DC: Government Printing Office, 1974.) 25 cents. [261]

World Health Organization. Technical Report Series, No. 581: Nonproprietary Names for Pharmaceutical Substances—Twentieth Report of the WHO Expert Committee. Pp. 32. Sw. fr. 6. No. 582: The Epidemiology of Infertility—Report of a WHO Scientific Group. Pp. 37. Sw. fr. 6. No. 583: Pregnancy and Abortion in Adolescence—Report of a WHO Meeting. Pp. 27. Sw. fr. 6. Promoting Health in the Human Environment. (A review based on the Technical Discussions held during the Twenty-seventh World Health Assembly, 1974.) Edited by Evelyn E. Meyer and Peter Sainsbury. Pp. 69. Sw. fr. 12. (Geneva: WHO; London: HMSO, 1975.) [261]

World Health Organization. WHO Offset Publication No. 22: Mental Health Services in Developing Countries. (Papers presented at a WHO Seminar on the Organization of Mental Health Services, Addis Ababa, 27 November to 4 December 1973.) Edited by T. A. Baasher, G. M. Carstairs, R. Giel and F. R. Hassler. Pp. ii+132. (Geneva: WHO; London: HMSO, 1975.) Sw. fr. 18. [271]

Department of Meteorology, University of Helsinki. Report No. 8: Diagnostic Studies on the Interaction Between the Time-Mean Flow and the Large-Scale Transient Fluctuations in the Atmosphere. By Eero Holopainen. Pp. 14. (Helsinki: Department of Meteorology, The University, 1975.) [271]

World Health Organization. Technical Report Series, No. 580: Control of Nutritional Anaemia with Special Reference to Iron Deficiency—Report of an IAEA/USAID/WHO Joint Meeting. Pp. 71. (Geneva: WHO; London: HMSO, 1975.) Sw. fr. 7. [281]

Carnegie Institution. Annual Report of the Director of the Department of Terrestrial Magnetism, 1974/1975. (Reprinted from *Carnegie Institution Year Book* 74.) Pp. 109-301. (Washington, DC: Carnegie Institution, 1975.) [281]

Science Council of Canada. Report No. 24: Technology Transfer—Government Laboratories to Manufacturing Industry. Pp. 61. (Ottawa: Information Canada, 1975.) \$1.20. [281]

Comptes Rendus des Travaux du Laboratoire Carlsberg. Vol. 40, No. 14: Ultrastructural Studies of Meiosis in Males and Females of the c(3)G17 Mutant of *Drosophila melanogaster* Meigen. By Soren W. Rasmussen. Pp. 163-174. Dkr. 11.50. Vol. 40, No. 15: Respiratory Rate Through the Growth-Division Cycle of *Acanthamoeba* SP. By Kirsten Hamburger. Pp. 175-186. Dkr. 11.50. Vol. 40, No. 16: Characterization of a Protein-Rich Beer Fraction by Two-Dimensional Immunoelectrophoretic Techniques. By Jørn Heigaard and Steen Bech Sørensen. Pp. 187-204. Dkr. 11.50. Vol. 40, No. 17: An Apparatus for Cultivation of Cell Suspensions in Continuous Flow Cultures. By K. H. Cohn and P. B. Suhr-Jessen. Pp. 205-213. Dkr. 11.50. (Copenhagen: Carlsberg Laboratorium, 1975.) [291]

Australia: Council of Scientific and Industrial Research. Report of the Division of Horticultural Research, 1973/1975. Pp. 137. (Adelaide: CSIRO, 1975.) [291]

Koninklijk Nederlands Meteorologisch Instituut. Mededelingen Verhandelingen. No. 95: On the Theory of the Electromagnetic Seismograph. By I. Csikos. Pp. 82. No. 96: Oceanography of the Ría de Arosa, N.W. Spain. By L. Otto. Pp. 210. (De Bilt: Koninklijk Nederlands Meteorologisch Instituut, 1975.) [301]

Great Britain

What's Science to History or History to Science? By Margaret Gowing. (An Inaugural Lecture delivered before the University of Oxford on 27 May, 1975.) Pp. 25. (Oxford: Clarendon Press; London: Oxford University Press, 1976.) 85p net. [301]

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